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THE WHITE COLD HAND

Physiologic, angiographic and pharmacologic study on the hand circulation with special reference to Raynaud's phenomenon

Birgitta Arneklo-Nobin

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Birgitta Arneklo-Nobin
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Abstract
The digital circulation was evaluated in normal healthy subjects by a finger plethysmographic method before and after local cold provocation at rest and with the measurements repeated after body warming and after body warming in combination with alcohol (the CCT-WA method). Differences related to age or sex were not found. Local cooling induced signs of vasoconstriction. However, further analysis showed that this response occurred only in tobacco smokers. This cold-induced vasoconstriction was not affected by inhibition of the sympathetic nervous system achieved through body warming, but it was eliminated after body warming plus alcohol orally. The observation suggests a special local vasodilatating mechanism for alcohol. The CCT-WA method can efficiently discriminate between a reduced circulation due to vasospasm or to organic obstructive changes in the vascular bed. Simultaneous registration of skin temperature increases further the efficiency of this discrimination. These possibilities for discrimination have considerable etiologic and therapeutic implications.

Isolated proximally located organic occlusions accessible to surgery can be diagnosed by the CCT-WA method and the indication for angiography can thereby be reduced. Angiography should instead be restricted to a preoperative descriptive examination and is preferably performed after body warming in combination with intraarterial injection of 4 mg of the α-receptor antagonist, phentolamine. In comparison with this antagonist, the NA-depleting agent, reserpine, was found to result in a more long-lasting release of local cold-induced vasoconstriction in the hand.

The contractile response to adrenergic agonists and 5-HT was investigated in isolated hand vessels. The quantitative pharmacologic analysis showed the existence of α-adrenergic and 5-HT receptors. This provides a rational pharmacologic counterpart to the therapeutic use of reserpine, and α-receptor blocking agents and 5-HT antagonists in Raynaud's phenomenon. The CCT method makes possible objective measurements of the effect of a given therapy.

Key words
Raynaud's phenomenon; Raynaud's disease; finger circulation; finger blood pressure; skin temperature; hand angiography; hand vessel receptors; reserpine.

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THE WHITE COLD HAND

Physiologic, angiographic and pharmacologic study on the hand circulation with special reference to Raynaud's phenomenon

by

Birgitta Arneklo-Nobin

Lund 1983

Upplagan är 700 exemplar

varav detta är nummer

To my children Rebecca and Christoffer

with all my love

This thesis is based on the following papers:

I. Raynaud's phenomenon in arterial obstructive disease of the hand demonstrated by locally provoked cooling.
Levin-Nielsen S, Arneklo-Nobin B, Hirai M, and Eklöf B.
Scand J Thor Cardiovasc Surg 12:105-109, 1978.

II. A method for the evaluation of finger circulation - with reference values for smokers and non-smokers.
Arneklo-Nobin B, Eklöf B, Levin-Nielsen S, and Sjöberg T.
Submitted.

III. Adrenergic and serotoninergic mechanisms in human hand arteries and veins studied by fluorescence histochemistry and in vitro pharmacology.
Arneklo-Nobin B, and Owman Ch.
Submitted.

IV. The differentiation of Raynaud's phenomenon by measuring skin temperature and digital arterial pressure and their responses to local cooling and to vasodilatation.
Arneklo-Nobin B, Norgren L, and Sjöberg T.
Submitted.

V. Indications for angiography and its optimal performance in patients with Raynaud's phenomenon.
Arneklo-Nobin B, Albrechtsson U, Eklöf B, and Tylén U.
Submitted.

VI. Reserpine treatment of Raynaud's disease.
Arneklo-Nobin B, Levin-Nielsen S, Eklöf B, and Lassen NA.
Ann Surg 187:12-16, 1978.

These papers will be referred to in the text by their Roman numerals.

ABBREVIATIONS

A	adrenaline
ABP	arm blood pressure
CCT	critical closing temperature
CCT-WA	critical closing temperature after body warming and alcohol administration
COMT	catechol-o-methyl-transferase
CTS	carpal tunnel syndrome
CWT	catheter to wrist circulation time
FSBP	finger systolic blood pressure
5-HT	5-hydroxytryptamine (serotonin)
MAO	monoamine-oxidase
NA	noradrenaline
SLE	systemic lupus erythematosus
TOS	thoracic outlet syndrome
TVD	traumatic vasospastic disease

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1. THE HISTORY OF THE WHITE COLD HAND

Maurice Raynaud published in the middle of the 19th century his work on "de l'asphyxie locale et de la gangréne symetrique des extremités" (82, 83). He presented a clinical syndrome consisting of sequential blanching, cyanosis and redness of the hands in response to a cold stimulus. He claimed that the symptoms had a symmetrical or bilateral distribution and that trophic changes were limited to the skin. Absence of organic arterial occlusions was another criterion, which he based on the existence of distally palpable pulses in the patients. He suggested that the symptoms were due to an increased "irritability" of the sympathetic nervous system. He was obviously deeply influenced by Claude Bernhard's recent discovery of the peripheral vasoconstrictive action of the sympathetic nervous system. Unfortunately, only a few of the patients he described in his work fulfilled his own criteria.

Instead of trophic changes limited to the skin, the skilful drawings in his book illustrated extensive gangrene, and information about distal pulses was not even reported for some patients. He is therefore himself to a large extent responsible for the still existing confusion about terminology and pathophysiology.

Already in 1901, Hutchinson (39) claimed that the criteria laid down by Raynaud did not describe a new isolated sympathetic disease but were rather symptoms caused either by sympathetically induced vasospasm or by organic changes in the vessels. He suggested instead the term Raynaud's phenomenon. This crucial observation on the dual pathophysiological mechanisms was, however, not immediately understood or accepted.

A reevaluation of the reports published until 1936 including a few hundred patients with so called Raynaud's disease, concluded that only 30 actually fulfilled all the criteria originally presented by Raynaud himself (38).

In 1932 Allen and Brown (5) made an important attempt to solve the terminological confusion with a critical review of Raynaud's publications. They found that only six patients of the 31 described by Raynaud fulfilled his own criteria. The remaining 25 patients probably had gangrene due to atherosclerosis, diabetes, scleroderma or thromboangiitis obliterans. They reintroduced Raynaud's original criteria and added another two characteristics: predeliction for women (9 to 1 in their own material) and absence of pain during an attack. Thirty years later Allen and collaborators (4) modified these criteria to the ones that today are most widely accepted to define Raynaud's disease: 1) white fingers evoked by cold or emotion, 2) symptoms usually bilateral, often symmetric, 3) seldom gangrene, 4) no other causative disease present, 5) symptoms more than two years.

Raynaud's disease has also been called primary Raynaud's phenomenon

(disease, syndrome) (14, 19, 90) or episodic idiopatic vasospasm (19) in order to stress the absence of known causative agents and for differentiation from secondary Raynaud's phenomenon. This latter term was introduced by Lewis and Pickering (58) to designate patients with a Raynaud's phenomenon with known etiology or with known causative agents. Presently the term is used in a more broad sense for description of white cold hands or fingers. Secondary Raynaud's phenomenon is associated with more than 20 different diseases or causes (Table I) (14, 19, 90).

Table I. Some major causes of secondary Raynaud's phenomenon

Occupational
Traumatic vasospastic disease (TVD)
Hypothenar hammer syndrome

Connective tissue disorder
Scleroderma - CRST
Dermatomyositis
Systemic lupus erytematosus (SLE)
Mixed connective tissue disorder
Rheumatoid arthritis
Polyarteritis nodosa

Obstructive disease
Arteriosclerosis
Thromboangiitis obliterans - "Buerger's disease"
Arterial emboli

Drug-induced
Ergot alcaloides
β-blocking drugs
Cytotoxic drugs

Neurological disorder
Central nervous system diseases
Thoracic outlet syndrome (TOS)
Carpal tunnel syndrome (CTS)

Miscellaneous
Cold agglutinins
Cold fibrinogen
Cryoglobulinemia

2. PRIMARY RAYNAUD'S PHENOMENON - RAYNAUD'S DISEASE

2.1. Prevalence and prognosis

The prevalence of patients with Raynaud's disease is unknown in the general population. In a healthy, indoor working, female population in Denmark, 22 % reported symptoms of cold sensitivity (72). Since the symptoms are often more socially inconvenient than disabling, the number of patients who seek medical advice is very varying. The percentage of patients with Raynaud's disease presented in different materials gives therefore little valuable information. The female dominance is obvious in all materials (19, 32, 89). Since the patients report that the symptoms improve during pregnancy, lactation and after menopause hormonal factors in the disease might be considered. Raynaud's disease has a favourable prognosis. In the impressive material presented by Gifford and Hines (32) an amputation was only necessary in 0.4 % of the patients. The disease will be less troublesome with increasing age and 46 % of the female patients improve spontaneously (32, for review see 90) often at the time of menopause. Raynaud's disease must therefore be regarded as a benign disease, a fact that must be thoroughly kept in mind when any form of therapy is taken into consideration.

2.2. Clinical characteristics

Beside the fulfilling of the criteria laid down by Allen and his collaborators in 1962 (4), patients with Raynaud's disease are often women younger than 40 years of age. They have lower skin temperatures (22-24 ^{o}C) than healthy subjects (29-32 ^{o}C), lower arm blood pressure and their finger blood flow is reported to be decreased (77). If these patients are provoked by local cooling, the time of rewarming to precooled temperatures is increased (57, 78, 86).

In patients with Raynaud's disease the cold-induced white fingers are distinctly well demarcated from the hand. It is also notable that the thumbs are never involved in this cold-induced reaction.

These well-demarcated white fingers observed after cold exposure has been attributed to closure of the digital arteries (26, 69, 71). It has, however, also been suggested that this process starts by cessation of flow in the venules and veins (22). The clinical background for this latter hypothesis is the observation that the Raynaud's disease often begins in young women with coldness, cyanosis and swelling of the fingers as the main manifestations (64). The very low pressures found in the capillaries during an attack were taken as an argument against the venous theory (53).

These theories are, however, not necessarily diverging. The symptoms described by Naide (64) resembles the symptoms defining acrocyanosis. Recently capillary flow velocity was regarded to be significantly decreased in patients with acrocyanosis, while the velocity was normal

in patients with Raynaud's disease (15). Finger flow measurements can not discriminate between patients with acrocyanosis and with Raynaud's disease (15). Since this was the method used at the time for the introduction of the "veno-constrictor theory", the above mentioned diverging theories can be due to an inability to disciminate between these two groups of patients. Acrocyanosis is presently seldom listed in materials of Raynaud's phenomenon but ten years ago it was a common diagnosis (19, 63, 75). This illustrates that even careful clinical criteria can not separate patients with different pathophysiologic reasons to their Raynaud's phenomenom.

The relevance of the pathophysiologic important veno-constrictor theory is dependent on the existence of vaso-constrictor mechanisms in the hand veins. Data on contractile receptor mechanisms in human hand veins are presented in paper III.

2.3. Pathophysiology

2.3.1. Sympathetic innervation of the upper extremity in healthy individuals

The sympathetic nervous system supports the upper extremity with vasoconstrictor and sudomotor nerves. The terminal fibres are located in the media-adventitial border just external to the smooth muscle layer (Fig 1).

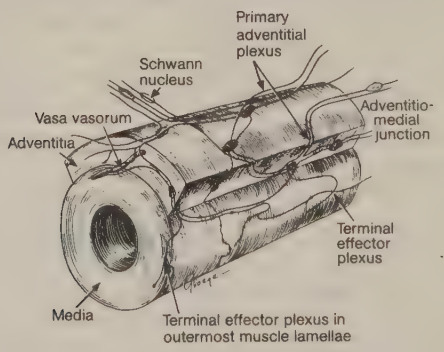

Fig. 1: Arrangement of the vascular neuroeffector apparatus. Post-ganglionic autonomic nerves ramify into small bundles forming a primary plexus, which is located in the loose adventitia.

The α-receptors of the effector cells are activated via the release of the transmitter substance, NA, from the nerve endings causing a vasoconstriction. The released NA can be eliminated either by mono-amine-oxidase (MAO) or by catechol-o-methyl-transferase (COMT). These enzymes are present in the arteries, but at least MAO seems to be absent in the veins (6).

On exposure to severe cold there is a marked increase in the affinity of the postjunctional α-receptors for NA which results in vaso-constriction. When an extremity is exposed to severe cold (0-10°C) for a prolonged time the initial vasoconstriction is followed by alternating periods of vasoconstriction and vasodilation, the so-called "hunting phenomenon" (55-57, 85). The phenomenon is greatly attenuated in the absence of sympathetic nerves and exaggerated in patients with Raynaud's disease (55-57). When the temperature of the tissues falls rapidly, sympathetic nerve conduction is interrupted and vasodilation occurs. This leads to an increase in flow, which rewarms the tissue and a new vasoconstriction can be induced (85).

There are quantitative differences between the cutaneous arteries and veins after cooling, in that the latter remain constricted at lower temperatures (85). This might partly be explained by the absence of NA destroying enzymes in the veins (see above).

Evidence for a differentiated sympathetic innervation of the upper extremity has recently been reported. Wallin and his collaborators (97) have shown that there are special fascicles in the sympathetic nerves of the upper extremity which react to temperature changes and emotional stress causing vasoconstriction in the skin, while there are other fascicles which react to a sudden drop in blood pressure causing vasoconstriction in the skeletal muscles (97). The nerve fibres leading to the skin are suggested to be anatomically located in close contact to the peripheral nerves, while those leading to the skeletal muscles are located close to the vessels (97).

The existence of vasodilating fibres in the upper extremity was suggested as early as 1931 (57). At that time this suggestion was based on the fact that there was a more pronounced increase in skin temperatures after body warming than after a blockade of the sympathetic nervous system. Body warming was assumed to activate vasodilator fibres. Such fibres have, however, never been demonstrated but β-adrenergic vasodilating receptors have recently been reported in arterio-venous (AV) shunts of the skin (20).

It is today obvious that vasoconstriction is not only caused by adrenergic sympathetic nerves but also by other vasoconstrictor factors (6, 35, 48, 65) and by inhibition of vasodilating mechanisms (20).

Concerning the relative importance of different vasoconstrictor mechanisms in hand vessels little knowledge is at present available.

Important fields to investigate are for example the 5-HT mechanisms and the mechanisms mediating vasoconstriction after local cooling (paper II, III, IV, V).

2.3.2. Increased sympathetic activity

The reduction in blood flow and skin temperatures registered in Raynaud's disease was originally - as mentioned above - regarded as due to an overactivity of the sympathetic nervous system (2, 82, 83). Indirect evidence for sympathetic overactivity was obtained by Coffman and Cohen (18). They used an isotope clearance technique and plethysmography to demonstrate that there was a reduction of total, capillary and AV-shunt blood flow in the fingers of patients with Raynaud's disease at rest compared to healthy subjects. This reduction in flow was, however, completely abolished after pre-treatment with sympathetic blocking agents (18).

Body warming has been regarded as an efficient inhibitor of peripheral vasoconstriction induced by the sympathetic nervous system (57, 86). The normal blood flow in skin and fingers achieved after body warming indicate that the arteries can properly dilate and consequently be absent of organic changes, which has also been confirmed by angiography of the hand (84). The general narrowing (basal vasospasm) of the vessels seen in these angiograms before vasodilatation can be eliminated after blockade of the sympathetic nervous system (84).

The intensity of this basal vasospasm does not correlate with the severity of the clinical symptoms but is rather an inconstant reflection of the response of hyperactive hand arteries to the angiographic procedural stress.

Angiographically visualized obstructions remaining after a sympathetic blockade represent organic changes (cf. 14, 37, 84). The prevalence of these organic changes do, however, correspond to the severity of the clinical symptoms and to the existence of skin lesions (59). This is in accordance with the opinion of Lewis and Pickering (58) that skin lesions should not be regarded as the result of uncomplicated spasm, but as being due to arterial occlusions.

This basic concept that episodic vasospasm is uncorrelated to skin necrosis, while organic changes in the vessels are necessary for the development of skin lesions is of great prognostic value. Patients with uncomplicated spasm must be informed to avoid iterating trauma to the fingers, which in this group of patients, easily can lead to organic changes in the vessels and thereby to skin necrosis.

Only a few years after the discovery of the sympathetic adrenergic transmitter noradrenaline (NA) in the middle of the 1950s several investigations were undertaken to find increased blood levels of the transmitter in patients with Raynaud's disease. Increased levels of NA were also reported in peripheral venous blood, and the suggested

explanation was a defect in the NA-destroying enzyme MAO (76). This suggestion was partly based on the fact that the skin temperatures in patients with Raynaud's disease are about 8°C lower than in healthy controls and that the lower temperature inhibits the enzyme systems. Decreased amount of MAO was also measured, but only in one patient. With more refined techniques diverging results on the NA blood levels in patients with Raynaud's disease have been obtained (14, 51, unpublished observations).

After local injection at the finger bases of 10^{-5}M - 10^{-7}M NA, vasoconstriction can be induced. This vasoconstriction is more pronounced in healthy controls than in patients with Raynaud's disease. This was taken as an indication of reduced sensitivity of the NA receptors which can be due to a sympathetic overstimulation in Raynaud's disease (68), a well-known reaction from other receptor studies (73).

Body cooling has the opposite effect (67) to body warming on sympathetic nervous discharge and general cooling thus leads to a significant decrease in hand blood flow both in healthy individuals and in patients with Raynaud's disease (55, 57, 67). The reduction in hand blood flow after body cooling is not influenced by additional local cooling in healthy subjects, whereas this extra cooling results in further decrease in blood flow in patients with Raynaud's disease (45). The sympathetically induced vasoconstriction performed by body cooling thus seems to be potentiated by a local mechanism in patients with Raynaud's disease provoked by local cooling.

The pathophysiologic mechanism causing vasoconstriction after local cooling is not known (see below) and even often neglected. Interesting information could be gained with a technique which could measure the effect of local cooling after inhibition of the sympathetic nervous system (paper II, IV).

2.3.3. "Local mechanisms"

During the 1930s Sir Thomas Lewis presented his impressive studies on Raynaud's phenomenon (55-58). He concluded that the vasoconstriction seen in Raynaud's disease was due to a "local fault" in the arteries themselves. He observed that a vasospastic attack could be provoked by local cooling even after blockade of the ulnar nerve, after body warming or after blockade of the sympathetic ganglion. He also noticed that if the hand was kept warm, body cooling could not provoke an attack. Thus, regardless of the degree of sympathetic activity, a typical Raynaud's attack could be provoked or prevented depending on the local digital temperature. Lewis made the interesting observation that after blockade of the ulnar nerve, local cooling could still induce a Raynaud's attack with cold fingers, but the skin was surprisingly quite red which could indicate that the capillaries were dilated (causing the red colour) while the digital arteries were constricted.

Nielsen later showed that, after anaesthesia of the finger base, local cooling could not induce any vasoconstriction (68). This has also been reported after blockade of the brachial plexus (75, 86). These experiments on nerve blockades at different locations indicate that vasoconstriction achieved after local cooling is dependent on nerve fibres in the brachial plexus. These fibres are probably not of sympathetic origin and they reach the hands along pathways separated from the ulnar nerve (cf. 97).

The discussion whether the vasoconstriction causing the white cold fingers seen in Raynaud's disease are caused by an overactivity of the sympathetic nervous system, by other neural mechanisms or by local non-neurogenic vasoactive mechanisms is far from completed. The relative importance if these different vasoconstrictive mechanisms is illustrated in papers II, III, IV, V.

Increasing interest has been focused on vasoconstriction caused by factors other than sympathetic activity. Halpern (35) reported elevated blood levels of 5-HT in patients with Raynaud's disease but these observations have not been confirmed (unpublished observations).

2.3.4. Increased viscosity

Pringle and collaborators (79) claimed that blood viscosity was doubled in association with an increase of fibrinogen in patients with Raynaud's disease compared to healthy controls. In subsequent studies these data have not been confirmed neither with regard to the increased levels of fibrinogen (45) nor to the enhanced viscosity (43).

2.3.5. Low blood pressure

Patients with Raynaud's disease are often reported to have a lower blood pressure than healthy controls. That this low blood pressure can be classified as a pathophysiologic factor is partly based on the observation that the patients' symptoms often start at an age when the blood pressure is low with impairment at an age when the blood pressure is higher (93).

3. SECONDARY RAYNAUD'S PHENOMENON

3.1. Traumatic vasospastic disease (TVD)

Traumatic vasospastic disease is most frequently reported among people working with pneumatic drills, chain saws, or grinding and polishing wheels for metal finishing, but it had also been reported among typists, pianists and sewing machine operators. Though scattered reports have appeared since 1918, most attention has been drawn to this condition during the last two decades (9, 14, 19, 40, 80, 81, 90). These vasospastic diseases are thus most common among workers using vibrating tools.

The most dangerous frequencies of vibrations from these tools have been reported to be 125 Hz but all frequencies above 40 Hz are injurious. Frequencies above 40 Hz stimulate the Pacini's corpuscles which are connected to the sympathetic nervous system. Hyvärinen suggested that the vasoconstriction registered in TVD was due to a sympathetic reflex integrated in the central nervous system after a prolonged overexcitation of the Pacinian corpuscles (40, 81). This continued sympathetic vasoconstriction has been suggested to cause the local hypertrophy of the arterial muscles seen in patients with TVD (9, 88). Once this hypertrophy is established, a normal sympathetic excitation creates a greater decrease in blood flow than would be the case in patients with a normal vessel wall.

Angiography performed in patients with TVD due to vibrations has shown severe vasospasm as well as organic changes (44, 81). The etiology of these organic changes is not known, but the character of the occlusions is probably benign, since skin necrosis is very rarely seen in these patients.

It is of great importance to evaluate the relative degree of vasospasm and organic changes in this group of patients since different therapies are available for these two conditions (paper IV).

Conn and his associates studied patients with Raynaud's phenomenon classified as TVD and found that the organic changes often included the ulnar artery (21). This artery is peculiarly vulnerable to trauma as it enters the palm distal to the carpal ligament and passes over the hook of the hamate. Further analysis showed that an isolated occlusion of the ulnar artery was only seen in special groups of workers, who did not use vibrating tools, but had iterated trauma towards the hypothenar region. They designated this condition "hypothenar hammer syndrome" (21). These patients are of great importance to select from other patients with vascular occupational trauma since their isolated ulnar arterial occlusion can be surgically removed and the patient thereby cured from their symptoms. In this study a method is presented which non-invasively can select these patients from other patients with TVD (papers IV, V).

3.2. Connective tissue disorders

The common feature for all these diseases is probably an immune dysfunction (3). The presence of circulating immune complexes has recently been demonstrated in systemic lupus erathematosus (SLE) and rheumatoid arthritis (for review see 14, 60). This is of pathophysiologic interest for the Raynaud's phenomenon seen in this groups of patients since the antigen-antibody complexes can cause smooth muscle contraction (95).

3.2.1. Scleroderma

Eighty to 90 % of patients with scleroderma have a Raynaud's phenomenon, which is the first symptom in approximately one third of cases (24). Raynaud's phenomenon can precede the other manifestations of the disease by more than five years. The etiology of scleroderma is still not known and the patients are therefore classified according to the clinical criteria suggested by Barnett (11) and later by American Rheumatologic Association (ARA) (60). There is evidence presented for abnormalities in the vessel wall and in the interstitial tissue as well as in the function of the sympathetic nervous system (90). Recently 5-HT has been proposed to play a pathophysiological role, being an important element in the collagen metabolism which can be linked to both the vascular and tissue fibrosis seen in scleroderma (unpublished report, 90).

Angiographically, vasospasm as well as multiple organ occlusive changes have been reported (54, 84). Special angiographic patterns for the disease have earlier been proposed but have been difficult to reconfirm. Dabich and collaborators studied 31 patients with scleroderma by angiography and plethysmography (23). Obstructions were seen in 26 of 27 patients. The ulnar artery was often more engaged than the radial artery. The blood flow was impaired in 70 % of the patients. Since angiography cannot separate these patients from other patients with Raynaud's phenomenon this examination ought to be abandoned in investigations of patients with scleroderma and replaced by non-invasive methods. Further support for this conclusion is the fact that these patients are not candidates for operation - which is the only indication for angiography - due to the spontaneous deterioration of the disease despite surgery (60).

3.2.2. SLE and rheumatoid arthritis

The frequency of Raynaud's phenomenon in these groups is 30-35 %. It has been concluded that the phenomenon seen in these patients is due to circulating immune complexes, which can cause contractions of vascular smooth muscles (95). Therefore, plasmaphoreses, has been reported to produce excellent results in these patients (50). The therapy must, however, be repeated with short intervals, is very expensive and ought therefore to be restricted to patients with severe symptoms.

3.3. Organic obstructive diseases

3.3.1 Arteriosclerosis

Angiography performed in patients with arteriosclerosis has confirmed that this group of patients has no increased vasoconstriction either at rest or after local cooling. The tendency for complete closure of the arteries after local cooling is, therefore, probably based on a normal, sympathetically induced vasoconstriction added to the diminished blood flow caused by the arterial obstructions (84).

3.3.2. Thromboangiitis obliterans - "Buerger's disease"

This disease was first described by Buerger in 1908 (16). Although some investigators have questioned its existence (99), it is obvious that, at least in the Far East, it is a more common disease than, for example, arteriosclerosis (37). The correlation of this disease to tobacco smoking is convincing but the real etiology is still unknown. Some authors claim that the etiology is persistent vasospasm while others point to organic obstructive changes (19, for review see 90). Support for the latter theory is the fact that an inflammatory infiltrate of all layers of the vessel is a prerequisite for the diagnosis. No adequate therapy is established, but the symptoms are reversible once the patient refrains from smoking (90).

The effect of smoking on peripheral vessels is not yet known. Nicotine has, however, been accepcted to induce vasoconstriction. The mechanism behind this vasoconstriction has recently been reported to be an inhibition of vascular prostacyclin formation without affection of the platelet tromboxane formation (98). This would lead to a relative increase of tromboxane, which increases the aggregation of platelets and induces vasospasm. Furthermore, even non-smoking people who are only exposed to cigarette smoke ("passive smoking") diminish their synthesis of prostacyclin (PGI_2) and show a decreased sensitivity of their platelets to antiaggregating prostaglandins (87).

Since there is the clear correlation between smoking and Buerger's disease it is of utmost importance for the understanding of this disease to evaluate thoroughly the effect of smoking on peripheral blood vessels (paper II).

3.4. Neurological disorders

The pathogenesis behind the Raynaud's phenomenon found in peripheral neurological disorders, such as carpal tunnel syndrome and thoracic outlet syndrome, is still under debate.

The white cold fingers seen in patients with thoracic outlet syndrome (TOS) were previously regarded to be due to a peripheral embolization from an aneurysm or stenosis of the subclavian artery caused by a compression (14). At present a compression of the brachial plexus with

its components of sympathetic nerve fibres is regarded to cause the peripheral vasoconstriction. This theory is, however, not proved and there is still no technique which can perform an objective assessment of this disease or its pathophysiology. Anyhow, an investigation of a patient with an acute onset of a localized Raynaud's phenomenon and TOS ought to include an angiography of the upper extremity for visualization of the subclavian artery (paper V).

4. REVIEW OF METHODS FOR THE MEASUREMENT OF FINGER CIRCULATION

In order to objectively evaluate the reduced blood flow causing Raynaud's phenomenon, methods have been developed which directly or indirectly record either the skin blood flow or the finger blood flow.

4.1. Measurement of skin blood flow

Measurement of skin temperature has been widely used for evaluation of the skin blood flow (57, 75, 86). When external conditions are kept constant, skin temperature is linearly related to skin blood flow until the skin temperature reaches 32-34°C (90). A further increase in skin temperature causes a more accelerated increase in skin blood flow. The major drawback of skin temperature measurement has been its poor reproducibility due to diurnal variations, especially among women (74), and the influence of the emotional state. Even in normal subjects repeated measurements show a coefficient of variation above 30 %. The above mentioned causes for temperature variations are both due to changes in sympathetic nervous discharge and can be controlled via methods and drugs influencing the sympathetic activity (17, 86).

Body warming, for example, has been shown to significantly improve the reproducibility in measuring skin temperatures (86). Lack of adequate increase in skin temperature to 32°C from a precooled state, after body warming indicates a reduced maximal skin blood flow capacity (Sigroth). This "temperature increase" has been reported to be a more reliable and reproducible parameter for evaluation of skin blood flow, than skin temperature measurements alone. The time of rewarming to regain the precooled temperatures after local cooling has also been a widely accepted parameter for evaluation of skin blood flow (86).

Other methods than skin temperature measurements for the evaluation of skin blood flow have recently been developed, i.e., heat-flow-meters, with promising results (92). An advantage with this method is that the probe used is heated, which means, that the measurements can be performed at the same skin temperature. This is thus a more simple method than body warming for vasodilatation of the skin vessels. The method can probably well perform an objective assessment of reduced skin blood flow but cannot discriminate between the reasons for this reduction.

Capillary blood flow has been measured by the radioisotope clearance technique (18) or estimated by capillaroscopy (14). The time-consuming and invasive radioisotope clearance techniques will probably be replaced by techniques such as the laser doppler technique (52) which currently in a simple and non-invasive manner evaluate the nutritional skin blood flow. This includes, however, only the arteries and venules located in the uppermost 2 mm of the skin.

All methods for evaluation of skin blood flow seem to have some limitations due to poor reproducibility and inability to differentiate between reduced blood flow due to arterial obstructions or to vasoconstriction. This can perhaps be explained by the fact that the sympathetic discharge is not controlled during the measurements.

4.2. Measurement of digital blood flow

For measurement of digital blood flow, plethysmography with venous occlusion, calorimetry and radio isotope clearance technique (18, 19, for review see 90) have been used, as have doppler ultrasound techniques; the latter being presently the most appreciated (91, 100). The pulse volume and contour have also been evaluated with different techniques (22, 78, 91), and special patterns for vasospastic and occlusive diseases have been reported. A minor limitation is, however, that these last mentioned methods cannot distinguish between arterial closure and pronounced vasospasm since the pulse pressure disappears at a finger blood pressure of 30-50 mm Hg.

4.3. Measurement of digital blood pressure

For evaluation of the digital blood flow, measurements of digital blood pressure are widely used. These measurements were markedly facilitated by the construction of a special finger blood pressure cuff (34). The technique has good reproducibility, and repeated measurements of finger systolic blood pressure (FSBP) showed only minor variations within and between days (5-10 %) (66). Simultaneous arteriography and FSBP measurements have shown that the cuff technique can be used to detect arterial obliterations in the finger arteries or in the palmar arcades (36, 37).

The importance of cold to induce a Raynaud's attack is almost the only causative factor that has not been questioned. It was therefore a great step forward in the objective evaluation of the cold induced vasoconstriction when a water perfusable finger cuff was constructed both for cooling the phalanx of the finger and for measuring the FSBP (36, 66, 67, 70). Patients with Raynaud's phenomenon reacted to local cooling with decreased FSBP, which was in some cases successively reduced to zero due to a complete closure of the arteries at a special temperature (CCT) (69).

The technique of local cooling of the finger seems to be superior to other cooling procedures not least since this local cooling - used in the CCT method - is performed under proximal arrest of the blood flow. Cooling of the hand in which flow persists creates temperature gradients along the arteries which introduce sources of error in the measurements (70). Standardization of the cooling procedure necessitates that the digital arterial temperature at the site of measurement is known. Local cooling during ischemia will make skin temperatures equilibrate with the tissue temperatures around the digital arteries in about five minutes (70).

In the present study the finger circulation in a number of patients with Raynaud's phenomenon is measured and evaluated by a new method, based on the conclusions about advantages and disadvantages which was drawn from the above mentioned techniques. Our method is a combination of an evaluation of the digital blood flow by measurements of FSBP before and after local cooling and evaluation of skin circulation by temperature registrations and rewarming rates. All procedures are performed before and after inhibition of the sympathetic nervous system (Paper II).

4.4. Angiography

It is obvious that the non-invasive methods are sufficient for most clinical considerations about changes in blood supply. However, when an exact localization of organic obstructions is needed, i.e., before surgery, angiography is a necessary diagnostic technique (26, 49, 62, 84).

The first angiogram was performed in 1896, but the technique was most appreciated and used during the 1950s and 1960s. Positive reports about the possibility of differentiating, not only between vasospasm and organic obstructive changes, but also between different diseases or causes of Raynaud's phenomenon, were presented (54, 59). With time the reports have been more critical to these possibilities. In contrast to angiographic examinations performed in the lower extremities, angiographies of the upper extremity are often complicated with severe vasospasm (7).

To minimize vasospasm in patient with Raynaud's phenomenon and thereby demonstrate organic changes in the peripheral arteries, general anesthesia, stellate block, pre- and perinjected vasodilatators, hand warming and oral alcohol have been used (26, 49, 51, 54, 84, 89, 90). The interpretation of the results indicate that the effect achieved after vasodilation is very individual. This can probably be explained by the existence of different reasons for vasospasm (14), which, however, seldom is analyzed.

Angiography revealed that normal subjects with or without arteriosclerosis do not have any vasospasm at rest (basal) (78). Local cooling of their hands induces some vasospasm but, interestingly, not in the thumbs.

Kent showed in 1976 (49) the absence of direct relations between the degree of vasospasm showed by angiography and the outcome of a sympathectomy. He concluded that angiographic examinations were of limited value as a guide to diagnosis or to management of Raynaud's phenomenon but would rather be restricted for visualization of organic changes available for surgery. The frequent use of brachial artery catheterization together with various types of trauma caused by, for example, vibrating tools have increased the incidence of complications such as emboli, stenosis or occlusions. This has created a new demand

for angiography of the hand, not least since the development of the microvascular surgical technique has made more peripheral areas available for surgery (62).

Angiography is time-consuming, expensive, potentially dangerous and uncomfortable for the patient, but necessary for a preoperative description. The disadvantages with the method must therefore be minimized. In the present thesis the indications for angiography of the upper extremity is presented with an evaluation of different vasodilating treatments and technical procedures used in order to optimize the angiographic procedure (Paper V).

5. REVIEW OF THERAPY

5.1. Sympathectomy

Digital ischemia was from the beginning suggested to be due to an overactivity of the sympathetic nervous system and consequently the logical treatment was cervico-thoracic sympathectomy (2, 33). With the increasing understanding of the multiple pathogenetic factors causing Raynaud's phenomenon in combination with an almost total lack of longlasting improvement after surgery (14, 47, 90), the use of sympathectomy has become more and more limited and is today almost abandoned. The only group of patients who is still reported to benefit from surgical sympathectomy are those who have a Raynaud's phenomenon due to a central nervous disorder (19). Since the indications for this group of patients are never threat of gangrene, the operation ought to be restricted only to those who have severe pain as a concomitant symptom in the cold-induced attacks.

Since the time when surgical sympathectomy was the only treatment for patients with Raynaud's phenomenon, these patients have continued to be referred to surgical clinics, though the treatments have successively been more pharmacological.

For pharmacologic and therapeutic purposes, the Raynaud's phenomenon - where the pathophysiological mechanisms are still often unknown - can be generally classified as being due to obstructive changes or to vasospasm.

5.2. Vasodilators

Vasospasm implies that the vasoconstriction is reversible. Therefore, vasospastic diseases ought to better respond to vasodilating therapy than diseases which cause ischemia due to organic changes. Vasodilators can either act directly by relaxing the blood vessel walls or by interfering with the action of the sympathetic nervous system (17). Treatments with vasodilators should in an optimal situation lead to pronounced peripheral vasodilation without concomitantly causing hypotension.

Previously the direct-acting drugs, i.e., papaverine and nicotine acid were used but by oral administration were found to be poor peripheral vasodilators and with side effects consisting of flushing, headache and gastrointestinal symptoms (17). The α-adrenergic receptor blocking agents have been reported to be beneficial for patients with vasospastic diseases but often cause a pronounced hypotension which is a great drawback since most patients with a Raynaud's phenomenon due to vasospasm have low blood pressures.

The recently introduced Ca^{2+}-blocking agents seem to be potent peripheral vasodilators and cause only moderate hypotension.

In this study data is presented on the vasodilating capacity of the α-receptor blocking agent phentolamine (cf. 7). Though this drug is only available for parenteral use, our data will point to the possibility to combine "α-blockers" with other treatments to achieve a more pronounced vasodilating effect (papers III, V).

5.3. Reserpine

Reserpine depletes the neurotransmitter NA from the sympathetic nerve endings, with an immediate vasoconstrictive effect, followed by vasodilation. Reserpine did not cause any vasodilation in normal individuals evaluated by angiography (51, 84), but an increase of the capillary blood flow without increase in the AV-shunt flow is reported (18).

Longterm oral therapy has been proved beneficial in some patients with Raynaud's phenomenon (1, 17, 51), but the side effects, especially night mares and depressions restrict the indications for this way of administration. Intraarterial injections of reserpine are reported to cause clinical positive results up to half a year after the treatment. In the present study we present an objective evaluation of the effect of one intraarterial injection of 0.5 mg reserpine. Due to the capacity of the drug to increase the capillary - nutritional - blood flow, this drug ought to be an excellent therapy for healing of ulcers, independent of etiology (paper VI).

5.4. Alcohol

It is generally believed that alcohol has a dilator action on the peripheral vessels. With large amounts of alcohol causing blood levels of ethanol above 0.2 % - which is often associated with intoxication (6) - such a vasodilation is due to direct depressive effects of ethanol on the central and autonomic nervous system. 120 ml 40 % ethanol decreased the drop of the gradient between systolic ABP and digital arterial pressure with 20 % in patients with Raynaud's disease, but this drop was, however, not significant (22).

Oral ingestion of smaller amounts of alcohol can cause vasodilation, vasoconstriction or biphasic actions on the peripheral blood vessels. Intraarterial injections of alcohol cause vasoconstriction (6). The same blood concentrations of ethanol can probably cause vasodilation when ethanol is given orally and vasoconstriction when given intraarterially.

It thus seems that alcohol exerts diverse effects on the peripheral blood vessels that is not only dose-dependent but may also be dependent of the route of administration.

In vivo techniques therefore seem to be superior to in vitro recordings to evaluate the effect of alcohol. In vivo techniques have also been used on mesenteric vessels and showed a depressive action

both after local or general application in both arterioles and venules (6). This was not due to an inhibition of the effect of serotonin or prostaglandins but rather to a direct effect on the smooth muscles. This direct effect can be due to an inhibition of the uptake and exchange of Ca^{2+} in the vessel smooth muscle cells (96). Another possible explanation is an effect on the MAO-function or a facilitating effect on the reuptake of the transmitter in the adrenergic nerves. Varying results concerning the interaction between alcohol and prostaglandins have, however, been reported. Kangasaho and collaborators have thus reported that platelet aggregation can be inhibited by ethanol in vitro or after prolonged ingestion. Even moderate ethanol intoxication significantly decrease the platelet tromboxane B_2, the clinically stable degradation product of the potent proaggregating and vasoconstrictive substance tromboxane A_2 (48).

In order to differentiate if a vasodilation is due to an inhibition of the sympathetic nervous system or to an inhibition of local vasoactive mechanisms, a method measuring the peripheral vasodilation both after body warming and after intake of alcohol would be valuable. In the present series, a method will be presented which fulfils these criteria (paper II).

5.5. Prostaglandin E_1 (PGE_1)

Some prostaglandins are potent vasodilators and have for years been used to achieve vasodilation during angiography (25). Recently prostaglandin E_1's effect on patients with severe forms of Raynaud's phenomenon has been presented. There was no improvement in patients without fingertip ulcera while 12 of 13 patients with ulcera improved (74). Noticeable is that the prostaglandin therapy was combined with antibiotics and surgical debridement in the latter group making the results somewhat difficult to interpret. Since PGE_1 is only available for infusion therapy and since the infusion must be performed at least during 72 hours, there is at present no convincing advantage of this treatment compared to a single dose of intraarterial reserpine.

5.6. Fibrinolytic enhancement

In scleroderma there may be fibrin deposits, intestinal proliferation and thrombosis in combination with reduced blood fibrinolytic activity (60). In patients with this manifestation anabolic steroids which enhance the natural fibrinolysis has been tried with impairment of symptoms (46). Concerning the Raynaud symptom in these diseases, anabolic steroids or cytotoxic drugs do not seem to be superior to intrarterial injections of reserpine for healing of finger ulcers. Preliminary results reported for the 5-HT receptor blocker ketanserin seem to be promising in patients with scleroderma (65).

6. AIMS OF THE PRESENT INVESTIGATION

The aim with the present study is to investigate:

1. the variation due to sex, age, smoking or local cooling in digital circulation in normal healthy persons

2. the existence of and difference in cold sensitivity between patients with Raynaud's phenomenon of different etiologies

3. the efficiency of the CCT method to measure the effect of a given therapy

4. the efficiency of the CCT method combined with measurements after vasodilating treatments (CCT-WA) to differentiate between Raynaud's phenomenon due to vasospasm or to organic changes

5. if the CCT-WA method combined with simultaneous registrations of skin temperature can further contribute to the distinction between different pathophysiological mechanisms causing a Raynaud's phenomenon

6. the indications for angiography of the upper extremity and to investigate which vasodilating treatment that will result in the most optimum visualization of organic vascular changes

7. the rational pharmacological basis for the use of vasodilating drugs influencing the adrenergic or serotonergic systems in diagnostic procedures and therapeutic trials by quantitative analysis of corresponding receptor mechanisms.

7. PATIENTS AND MATERIAL

Sixty-eight healthy volunteers were investigated with the CCT and the CCT-WA techniques. Fifteen of these served as controls for the patients presented in paper I. These volunteers were members of the laboratory staff and patients with minor non-vascular diseases.

Fifty-three healthy volunteers were investigated for evaluation of the CCT-WA technique (see below) presented in paper II. These subjects were divided into three age groups: ≦ 30 years (10 women, 10 men), > 30 ≦ 45 years (10 women, 10 men), < 45 years (8 women, 5 men). Twenty-nine were non-smokers (12 women, 17 men) and 24 were smokers (16 women, 8 men). In this paper the volunteers were not restricted to indoor workers. All were free from symptoms of white cold fingers or suspicion of cardiovascular or connective tissue disorder.

Twenty-seven patients with Raynaud's phenomenon were investigated by the CCT technique in paper I. Seven of these patients had Raynaud's disease, 5 patients had subclavian stenoses and 15 patients had thromboangiitis obliterans all with angiographically visible arterial stenoses or occlusions. All patients with Raynaud's disease were females while all, except two, patients with thromboangiitis obliterans were men.

One hundred and twenty-three patients with Raynaud's phenomenon of six different etiologies were examined with the CCT-WA technique in paper IV. The phenomenon was due to traumatic vasospastic disease (TVD) in 50 patients, to hypothenar hammer syndrome in 10 patients, to scleroderma in 18 patients, to primary Raynaud's phenomenon (Raynaud's disease) in 18 patients, to carpal tunnel syndrome (CTS) in 10 patients and to previous skin trauma in seven patients. All patients in the groups with TVD and hypothenar hammer syndrome were men, while all patients in the groups with primary Raynaud's phenomenon and carpal tunnel syndrome were women.

Five more patients with primary Raynaud's phenomenon (Raynaud's disease) were investigated in paper VI with the CCT-technique for the objective evaluation of the medical treatment.

Eighty-five patients with Raynaud's phenomenon were examined with angiography. Twenty-seven patients (paper I) were examined in order to determine the localization of the suspected arterial obstructive changes and five patients (paper VI) were angiographied in order to objectivate the result achieved half an hour after intraarterially administered reserpine.

The remaining 53 patients are presented in paper V and they were angiographied in order to evaluate the effect of six different vasodilating therapies given to optimize the angiographic visualization of organic changes. The results obtained from 25 of these patients are

also included in paper IV for comparison with the results obtained with the CCT-WA technique.

For the analyses by fluorescence histochemistry and in vitro pharmacology presented in paper III, small segments of hand arteries and veins were removed from 60 patients during hand operations due to CTS or to arthrosis.

8. METHODS

All investigations with the CCT- and CCT-WA techniques (presented below) (papers I, II, IV and VI) were performed with the patients in supine position at a stable room temperature. The patients had refrained from smoking during the 3-4 hours prior to the examination. The ABP was measured bilaterally using a 12 x 28 cm rubber cuff.

8.1. The CCT method

The FSBP was measured (paper I) in all fingers (66, 70) using a plastic cuff of 2.5 x 10 cm on the mid phalanges of the four ulnar fingers and on the proximal phalanx of the thumb with mercury in rubber strain gauges for volume detection on the pulps. Signals from the gauges were recorded on a plethysmograph (Medimatic, model SB 2, Copenhagen). The FSBP was recorded in one finger during local cooling of the mid phalanx by gradually decreasing temperature (70). With the finger blood flow stopped by a tourniquet of the proximal phalanx local cooling was performed for 5 minutes with a specially devised plastic cuff which also allowed measurement of FSBP (Fig 2).

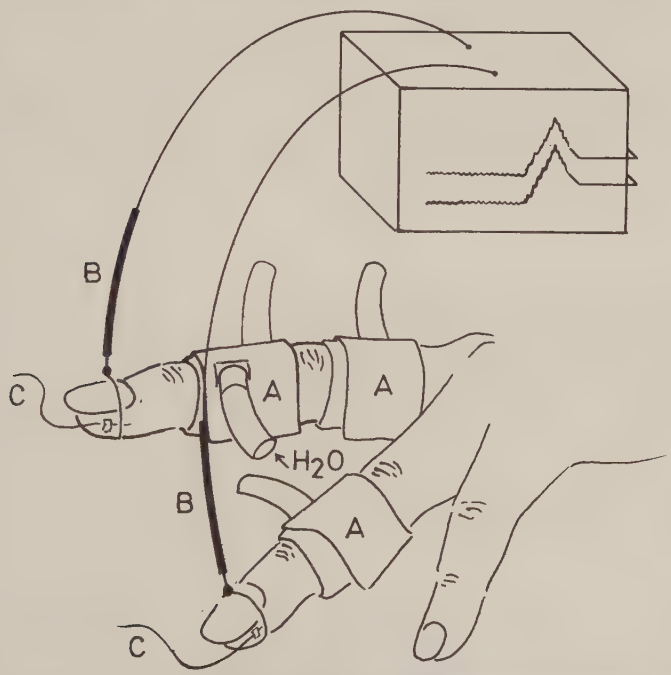

Fig 2. Schematic drawing showing the equipment with the placement of the tourniquets (A), the strain-gauges (B) and the thermocouples (C). For further explanation see Method.

Determinations of FSBP were repeated after local cooling at 5°C intervals down to about 7°C in healthy volunteers and down to 10°C in patients with Raynaud's phenomenon. The cold-induced decrease in FSBP was calculated. FSBP was also measured in an adjacent finger in order to register any general blood pressure change during the cooling procedures.

At a special temperature the FSBP decreased to zero indicating a complete closure of the arteries and this temperature is called the critical closing temperature (CCT). The CCT levels were registered for each patient.

8.2. The CCT-WA method

The CCT method was further developed (paper II) with repeated measurements after body warming for 15 minutes by an electric pad placed over the abdomen (50°C). The measurements were also repeated after continuous body warming 15 minutes after oral intake of 30 ml of 40 % ethylalcohol. To the FSBP measurements performed (see above CCT method) were also added recordings of fingertip temperatures of all fingers bilaterally at the start of the investigation, after the 15 minutes of body warming and then after the further 15 minutes' body warming in combination with alcohol intake. Measurements of the rewarming rate (expressed as centigrades per minute) was calculated from the first 10 minutes after the local cooling was finished and the cuffs were removed. In the present study we introduce the variable rewarming rate instead of total time of rewarming earlier used, which has been reported to be markedly tedious in many pathological groups (> 30 min) (77, 86).

This extension of the original CCT method has been designated the CCT-WA method.

Body warming alone or in combination with alcohol intake is used to differentiate between a decrease in finger circulation due to vasospasm or to organic obstructive changes. After eliminating vasospasm, any decreased values obtained ought to be due to organic obstructive changes. Determinations of skin temperature and rewarming rates were added to make a more sophisticated pathophysiological classification possible (papers II, IV).

The following variables were measured and evaluated in papers II and IV: a) fingertip temperatures for the most severely affected finger, the control finger and mean values for all fingers of each hand separately before and after body warming and after body warming plus alcohol; b) ABP, c) FSBP of the most severely affected finger and the control finger; d) the FSBP/ABP ratio and the FSBP 10°C/FSBP ratio for calculation of e) a reaction value to cold (CRV) defined as FSBP 10°C/FSBP corrected for a possible change in blood pressure evaluated from FSBP of the control finger. CRV value of 1 means no change in FSBP after local cooling and a CRV of 0 means a complete closure of

the arteries after local cooling to a given temperature, the CCT. Finally, f) the rewarming rates after local cooling are presented.

8.3. Angiography

All angiographies presented in papers I and VI were performed with the contrast medium meglumine metrizoate (280 mg iodine per ml). In paper V 10 patients were examined with meglumine metrizoate and these patients were compared to 42 other patients who were examined with metrizamide (300 mg iodine per ml), a contrast medium of low osmolality. The first three of these 42 patients were examined with both contrast media. All angiographies were performed from the femoral artery in order to visualize proximal organic changes and to avoid vasospasm, which otherwise could be the result of a local puncture of the brachial artery.

A pigtail catheter (ID/OD 1.4/2.3 mm) with multiple side holes was placed in the ascending aorta, whereafter 3 000 IU of heparin was infused into the catheter. Aortography was performed by injection of 40-50 ml of 60 % contrast medium at a rate of 25 ml per sec. The catheter was then exchanged for a similar catheter shaped for selective work with only one end hole, and catheterization of the brachial artery was carried out. Ten ml of contrast medium was injected at a rate of 7-8 ml per sec and films were exposed over the upper and lower arm to cover the arterial phase. Appropriate delay of exposure for examination of the hand was calculated from these films. Examination of the hand was made after injection of 20 ml of contrast medium in the brachial artery at the rate of 7-8 ml per sec and 1 film per sec was exposed for 10 sec. From the angiograms the CWTs were calculated.

After the initial angiography was performed 5 different vasodilating procedures were tested alone or in combination: (see Table II) 1) an axillary blockade of the brachial plexus by injection of 15-20 ml 2 % lidocain (Xylocain®, Astra), with an angiography performed when the maximal effect of the blockade was registered; 2) 0.5 mg reserpine (Serpasil®, Ciba-Geigy) dissolved in 10 ml NaCl slowly injected intraarterially, with angiographies performed 10, 20, 30 and 40 minutes after the injection; 3) 2, 4 or 6 mg of the α-receptor antagonist phentolamine (Regitin®, Ciba-Geigy) intraarterially; 4) body warming, by placing a warming pad of 50°C over the abdomen and covering the patient with blankets for 15 min whereafter angiography was performed; 5) 30 ml of 40 % alcohol orally in combination with body warming for 15 minutes.

The effectiveness of the vasodilating procedures was tested by letting the patient hold his or her hand in a bowl with ice-cubes for 5 minutes, a procedure known to induce vasoconstriction. An angiography was performed before and after this cooling procedure. If more than one vasodilating treatment was tested in the same patient new angiographies were performed and in most patients also angiographically controlled local cold provocation.

Table II. Vasodilatating treatments in 52 patients with Raynaud's phenomenon.

Group	n	Treatment
1 A	9	Blockade of the brachial plexus + local cooling
1 B	4	Blockade of the brachial plexus
1 C	4	Blockade of the brachial plexus + local cooling + reserpine
2 A	6	Phentolamine + local cooling
2 B	5	Phentolamine
2 C	2	Phentolamine + local cooling + blockade of the brachial plexus + local cooling
2 D	3	Phentolamine + local cooling + reserpine
3 A	9	Body warming + local cooling + phentolamine + local cooling
3 B	4	Body warming + phentolamine
3 C	4	Body warming + local cooling + orally administered alcohol + local cooling
3 D	2	Body warming + local cooling + reserpine

8.4. Vascular preparations for studies by fluorescence histochemistry and in vitro pharmacology

For paper VI the vessels were removed during hand-operations under blood-free conditions. Short segments were dissected from small contributions of the radial artery to the thenar musculature, from the anterior interosseal artery, and from hypothenar branches of the ulnar artery, as well as from the associated veins. Attempts were made to remove vessels with a diameter less than 1 mm. Immediately after removal the vessels were transferred to an ice-cold Krebs-Ringer-glucose solution and transported to the laboratory. Within 1 hr, a 5 mm long piece was mounted between two L-formed metal holders in a 50 ml manteled organ bath for recording of circular motor activity (27, 28) by force-displacement transducers, recording being performed with a Grass 79D polygraph.

Pieces of the excised vessels were frozen in propane-propylene at the temperature of liquid nitrogen, freeze-dried, and further processed for fluorescence microscopic demonstration of catecholamines according to the formaldehyde method of Falck and Hillarp (13, 29, 30). Small pieces of the vessels were also fixed in Bouin's solution and transverse paraffin sections of 5 µm thickness were stained in hematoxyline and eosin. These were used in order to distinguish arteries from veins, which was sometimes difficult during the blood-free operation, and for measurement of the vessel dimensions. Finally vessels were also homogenized in ice-cold perchloric acid for fluorimetric determinations of NA levels (12, 41).

9. STATISTICS

Statistical evaluation was performed with parametric methods and testing was done with Student's t-test in papers I and III. In papers II and IV data were analysed by classical analysis of variance. Parametric statistics were thereafter also performed with Student's t-test using levels of significance, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

10. RESULTS AND DISCUSSION

10.1. Finger circulation in healthy subjects (papers I, II)

No significant differences in finger circulation between sex or age groups were found. This was somewhat surprising, but could be explained by the hard inclusion criteria for the control group, which excluded many proposed individuals. There was, however, a significant difference in reaction of finger circulation to local cooling in smokers and non-smokers. In the present study all data are therefore presented for the whole group of healthy subjects as well as separately for smokers and non-smokers (paper II).

10.1.1. Skin temperature (paper II)

The skin temperature (mean value for all five fingers) was at rest 31.7 ± 3.5°C (mean ± SD) (range 25-36°C). After body warming the temperature increased to 33.7 ± 2.5°C ($p < 0.001$) with a further increase after body warming plus alcohol intake to 34.5 ± 1.4°C ($p < 0.001$). These increasing temperatures in combination with decreased SD:s are in good accordance with previous reports showing that measurements of skin temperature are only of value and reproducible if the influence of the sympathetic nervous system is inhibited (86). Body warming is widely accepted as an efficient inhibitor of sympathetically induced peripheral vasoconstriction in the skin (57, 75, 86). The skin temperature values obtained after body warming have earlier been reported even to be higher than those achieved after blockade of the sympathetic nerves (57). This indicates that the use of body warming is not only markedly efficient to inhibit sympathetically induced vasoconstriction but also easy to repeat and comfortable for the patient (paper II).

It is well-known that skin temperatures have a linear relationship to skin blood flow below 32-34°C, but above this level marked increases in skin blood flow may result in only a small increase in skin temperature (75, 90). Measurements of skin temperatures alone may therefore not be sufficient to evaluate the skin blood flow. We therefore added calculations of the rewarming rate after local cooling which we introduced instead of time of rewarming which is previously reported to be a reproducible and relevant but time-consuming parameter for evaluation of skin blood flow (78). The rewarming rates increased with 40 % after body warming and with further 20 % after the addition of alcohol.

There were no significant differences in skin temperatures between smokers and non-smokers, though there was a tendency for lower values in smokers before body warming. These findings are in good accordance with earlier reports (86).

10.1.2. Finger systolic blood pressure (FSBP) (papers I and II)

The FSBP values found were unchanged after body warming and after body warming plus alcohol. The FSBP/ABP ratio was around 1 for the third finger where the FSBP was measured by the water-filled cuff, but significantly lower (5-7 %) in the fourth finger where the FSBP was measured by the air-filled cuff. This discrepancy is probably an effect of the recording system.

The FSBP/ABP ratio was not significantly altered after body warming or after body warming plus alcohol. This indicates that there is no sympathetically induced vasoconstriction at rest in normal hand vessels which is in agreement with a previous suggestion based on angiographic studies (84).

After local cooling to 10°C the FSBP decreased with about 5 % in the healthy subjects in paper II and with about 10 % in the controls in paper I. This discrepancy cannot be fully explained but might be due to the more heterogenous control group in paper I. The FSBP 10°C/FSBP ratio was unaffected after body warming but increased after body warming plus alcohol administration. The values obtained after local cooling provided three interesting bits of information (paper II) (Fig 3): 1) the decrease in FSBP after local cooling was accounted for by the smokers, 2) the decrease in FSBP was more pronounced at 15°C than at 10°C, 3) the decrease in FSBP in smokers was not altered after body warming but was abolished after body warming plus alcohol. This could either indicate that smokers have a higher sympathetic discharge to the digital arteries being finally abolished by alcohol or that alcohol has local effects on the cold sensitivity. The first suggestion is, however, partly contradicted by their normal skin temperatures.

The observations that the decrease in FSBP after local cooling is more pronounced in smokers than in non-smokers and that this decrease is abolished after addition of alcohol have not earlier been reported. Smoking has been found to increase the aggregation of platelets and also to cause a relative increase in the blood levels of tromboxane B_2 (87, 98). There are indications that alcohol reduces the aggregation of platelets, interferes with the prostaglandin synthesis (48) and the Ca^{2+} metabolism (96). Further studies have to be performed on this notable effect of alcohol, not least since the same capacity of alcohol to abolish cold induced decrease in FSBP was found in patients with primary Raynaud's phenomenon (paper IV).

A more pronounced vasoconstriction at 15°C than at 10°C has earlier been found in patients with TVD examined by the CCT method. This tendency was explained by the hunting phenomenon (cf. 85, 94). With a local cooling of 5 minutes, as used in the present method, the hunting phenomenon ought, however, to be avoided and can therefore not be the only explanation. However, we found this increased cold sensitivity at 15°C not only in tobacco smokers but also in patients with TVD and in

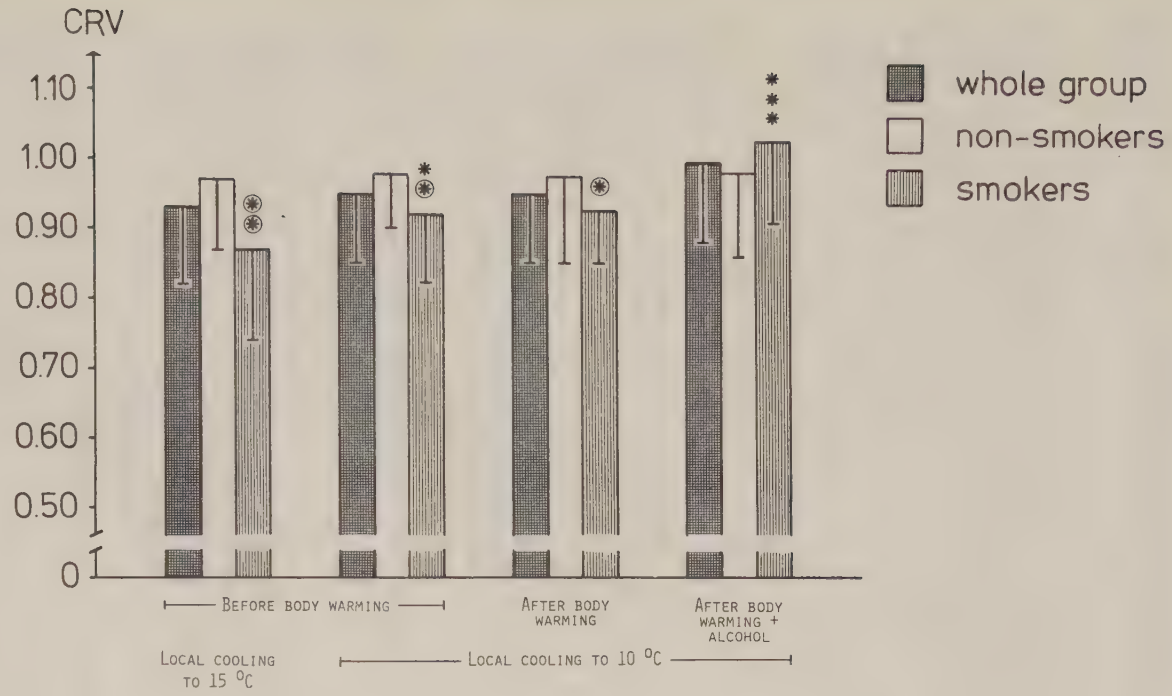

Fig 3. The quotients between FSBP after local cooling to 15°C and 10°C compared to FBSP before local cooling (when the quotients are corrected for variation in FSBP of the control finger = CRV). The cooling procedures are repeated after body warming and after body warming plus alcohol. Mean values + SD. Significant differences between smokers and non-smokers are denoted by ⊛ $p < 0.05$, ⊛⊛ $p < 0.01$. Significant differences from preceding test procedure are denoted by * $p < 0.05$, *** $p < 0.001$.

patient with primary Raynaud's phenomenon (paper IV). It is possible that temperatures around this level induce some local vasoactive process which is still unknown.

10.1.3. Concluding remarks (papers I and II)

The present investigation shows that body warming significantly increases the skin temperatures and the rewarming rates. This indicates that under resting conditions there is a basal vasoconstrictor sympathetic tone in the skin vessels. Body warming does not affect the FSBP, FSBP/ABP and FSBP $10^{\circ}C$/FSBP indicating that there does not exist any basal or local cold induced vasoconstriction mediated via the sympathetic nervous system. Body warming in combination with alcohol abolishes the cold induced decrease in FSBP, a decrease which is only seen in smokers and is more pronounced at $15^{\circ}C$ than at $10^{\circ}C$. This suggests that alcohol acts by another mechanism than simply by inhibiting sympathetic vasoconstriction.

10.2. Innervation and contractile mechanisms of hand vessels (paper III)

Fluorescence microscopic analysis of hand vessels following the formaldehyde reaction for demonstration of biogenic amines demonstrated a well-developed sympathetic nerve supply of both arteries and veins. The innervation was best developed in the arteries where it formed a network of fibres located in the adventitia, superimposed on the smooth muscle media layer. The musculature in the wall of the veins was less well developed and demarcated, and here the adrenergic nerve terminals coursed in among the superficial smooth muscle cells. This arrangement of innervation is characteristic for the autonomic nerve supply to vascular beds (e.g. 73).

The results of the fluorescence histochemistry of the adrenergic nerve supply were consistent with the fluorometric determination of tissue noradrenaline.

The contractile response of hand arteries and veins to various agonists was studied by cumulative application under standardized conditions, including blockade of neuronal and extraneuronal amine uptake mechanisms in order to allow for quantitative evaluation (42). The relative potency based on the ED_{50} values (i.e. dose giving half maximum response) for the sympathomimetic contraction was in both arteries and veins in the order A > NA > isoprenaline which is characteristic for α-receptor mediated response.

The α-receptor antagonist, phentolamine, shifted the dose-response curve of NA in both arteries and veins towards higher agonist concentrations. Schild plots (8) gave a straight line with a slope not significantly different from unity, confirming the competitive nature of the phentolamine-induced inhibition of the contraction. Phentolamine had an affinity (expressed in terms of the pA_2 value) for

the contractile receptor of the hand vessels that agreed well with the affinity for corresponding receptors in other smooth muscle tissues (31).

5-HT had a relative potency on the hand arteries, as evidenced from the ED_{50} values, which was the same as that of A and NA. In the veins it was significantly higher than that of both catecholamines. Ketanserin, a 5-HT antagonist (65), inhibited the 5-HT response in a competitive probably also irreversible manner in arteries (Fig 4) whereas in the veins, ketanserin blocked the 5-HT induced contraction in a non-competitive way.

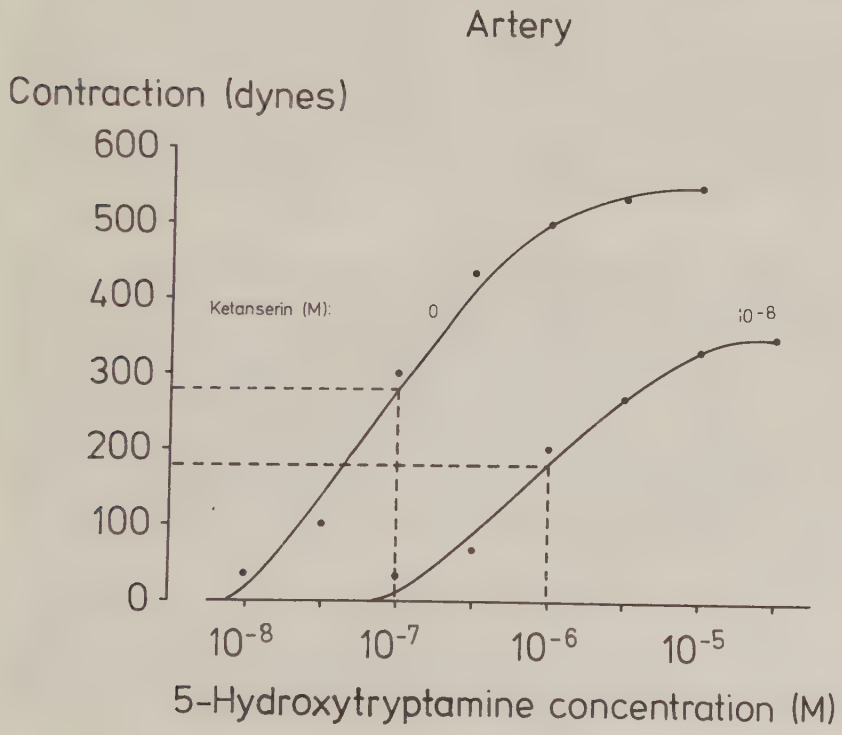

Fig 4. For explanations see above.

10.2.1. Concluding remarks (paper III)

We have demonstrated a dense network of NA-containing sympathetic nerve fibres innervating hand arteries and veins. We have also characterized α-adrenergic receptors in hand arteries and veins and 5-HT receptors in hand arteries. These receptor findings provide a rational pharmacological counterpart to the therapeutic use of α-receptor antagonists, reserpine and the 5-HT antagonist ketanserin, in peripheral circulatory disorders such as Raynaud's phenomenon.

10.3. Pathophysiological differentiation of Raynaud's phenomenon (paper I, IV)

All patients with Raynaud's phenomenon presented in paper I showed a significantly lower FSBP than ABP. Among these patients those with primary Raynaud's phenomenon and with thromboangiitis obliterans had similar FSBP values at rest. After local cooling, however, the reduction of FSBP was more pronounced in patients with primary Raynaud's phenomenon than in patients with thromboangiitis obliterans. Patients with primary Raynaud's phenomenon were found to have an increased arterial tone starting at about 24°C compared to the control group with a complete closure of the arteries after local cooling at 18.5°C. The patients with thromboangiitis obliterans started to have an increased arterial tone at 16.5°C and their critical closing temperature was 11°C.

Cold sensitivity in patients with primary Raynaud's phenomenon and thromboangiitis obliterans may have different pathophysiological mechanisms. The cold-induced decrease in FSBP seen in patients with Raynaud's disease is probably due to a pathological cold-induced vasoconstriction while in patients with thromboangiitis obliterans it is due to a normal cold-induced vasoconstriction acting on vessels with organic changes. Support for this theory can be gained from results obtained from measurements following repeated cold provocations. It appears that a certain amount of time must elapse before the same temperature can induce a closing attack in patients with primary Raynaud's phenomenon while in patients with thromboangiitis obliterans the attacks could be repeated each time the temperature is lowered to the same level.

In paper IV we evaluated the skin and digital arterial circulation in 6 different groups of patients with Raynaud's phenomenon. The FSBP, the FSBP/ABP and FSBP 10°C/FSBP ratios were normal only in two patient groups (Figs 5 and 6): patients with CTS and patients who have had a skin trauma. Pathologic values were, however, obtained in these groups for skin temperatures and rewarming rates.

In patients with CTS the skin temperatures were normal at rest, decreased after body warming and returned to normal values after body warming plus alcohol. The rewarming rate was significantly decreased at rest and after body warming (Arneklo-Nobin, Rosén, Thorngren,

Werner, in manuscript). In patients with previous skin trauma the skin temperatures were markedly low (22.4±0.3°C) and remained at the same level after body warming and after body warming plus alcohol administration. The rewarming rates were also significantly decreased with only small improvement after body warming and body warming plus alcohol. The normal FSBP values achieved in these two groups of patients showed that their Raynaud's phenomenon would not have been diagnosed with the original CCT method. Some patients with Raynaud's phenomenon can obviously not be diagnosed without additional measurements of skin circulation, which must also be repeated after inhibition of the sympathetic nervous system. The remaining low skin temperature values obtained for the skin trauma group even after sympathetic inhibition, indicate an organic change, which can well correspond to the clinical picture of direct trauma towards the skin. Complementary studies to explain the skin temperature reactions seen in CTS will soon be published (in manuscript, see above).

The reduction in skin circulation causing a Raynaud's phenomenon has earlier been regarded to be secondary to a decrease in the digital arterial circulation. The results obtained in the present study with normal FSBP values but pathologic skin temperature reactions can indicate that the skin circulation is regulated by mechanisms which are separated from those acting on the digital arteries. This theory is in accordance with previous findings of a differentiated sympathetic innervation of the hand vessels (97) (cf. 2.3.1.).

For the remaining patient groups in paper IV there were changes in the FSBP, the FSBP/ABP and/or in the FSBP 10°C/FSBP ratios (Figs 5 and 6). This indicates that the digital arterial circulation in these groups is affected. Patients with a hypothenar hammer syndrome who by definition have a complete closure of the ulnar artery, had a reduced FSBP and a reduced FSBP/ABP ratio (< 0.8) which was unchanged after body warming and body warming plus alcohol, indicating organic changes. Since they did not react to local cooling with any further decrease in FSBP, this occlusion was probably not located in the digital arteries since this localization would have given a flow reduction which should have led to complete closure after local cold provocation (37). The combination of a markedly reduced FSBP/ABP ratio and without further decrease in FSBP after local cooling independent of vasodilating procedures was only characteristic for patients with hypothenar hammer syndrome.

A FSBP/ABP ratio less than 0.8 and with a further decrease to zero was seen in patients with TVD and scleroderma. Multiple organic occlusions were showed angiographically in some of these patients. A FSBP/ABP ratio of less than 0.6 always indicates multiple occlusions and was in our material seen only in patients with scleroderma (Fig 6). The cold-induced decrease in FSBP seen in patients with TVD and scleroderma was significantly improved after body warming, indicating that a sympathetically induced vasospasm was reversed. Since there was a remaining marked decrease in FSBP after body warming and body warming plus alcohol compared to controls, this points to the presence also of

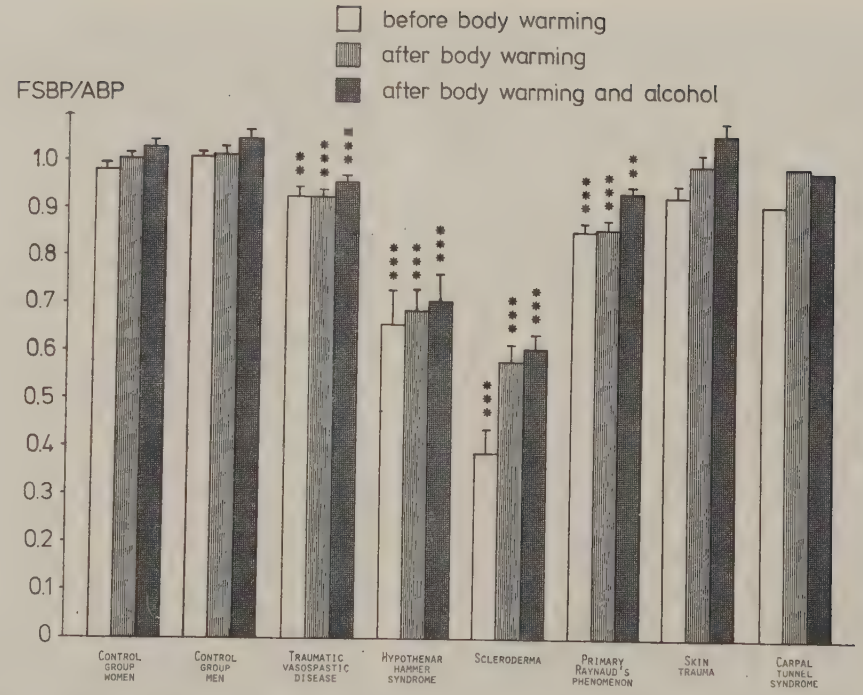

Figure 5. Effect of body warming and body warming in combination with alcohol on FSBP related to arm blood pressure (FSBP/ABP) for different groups of patients with Raynaud's phenomenon and control groups.

Each value represents the mean ± SEM. Significant differences from the control group are denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

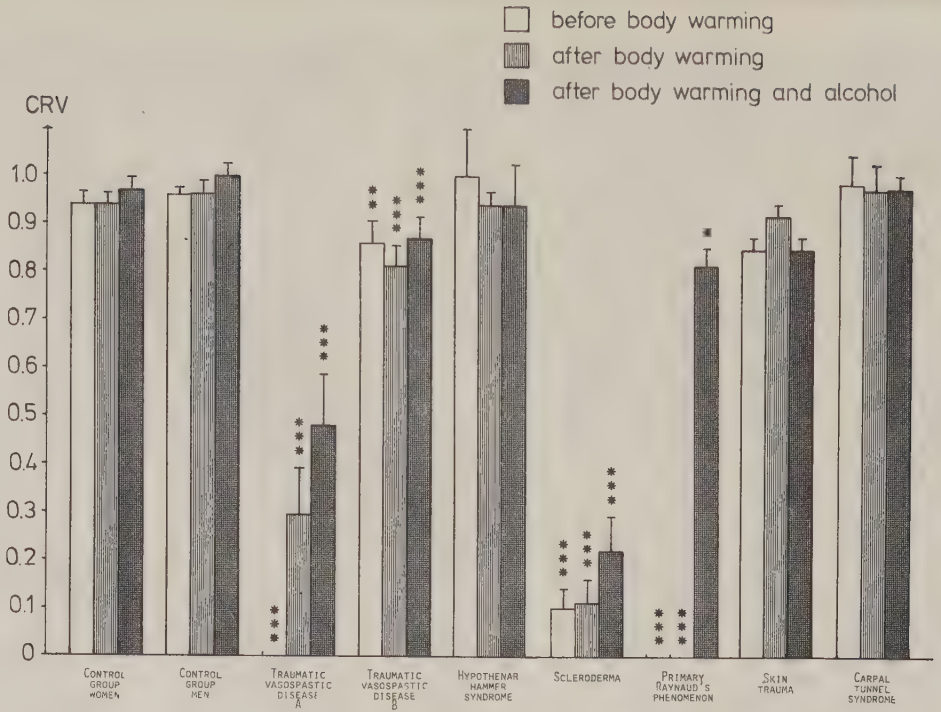

Figure 6. Effect of body warming and body warming in combination with alcohol on the CRV for different groups of patients with Raynaud's phenomenon and control groups. (CRV = $FSBP_{10^oC}/FSBP$ correlated to changes in FSBP of the control finger.)

Each value represents the mean ± SEM. Significant differences from the control group are denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

organic obstructive changes in these patient groups. Skin temperature measurements discriminated well between patients with scleroderma and patients with TVD in that skin temperatures were markedly lower and unchanged after vasodilating treatments in patients with scleroderma.

Patients with primary Raynaud's phenomenon also had a marked decrease in FSBP after local cooling causing a complete closure of the arteries at 15-17°C. This cold-induced decrease was not affected by body warming but was almost completely abolished after body warming together with alcohol administration.

10.3.1. Concluding remarks (papers I and IV)

With the CCT technique there was a marked cold-induced decrease in FSBP after local cooling in patients with primary Raynaud's phenomenon, subclavian stenosis with peripheral embolization and in patients with thromboangiitis obliterans. The cold sensitivity was, however, more pronounced in patients with primary Raynaud's phenomenon and probably reflects a pathological cold-induced vasospasm in this group of patients, in contrast to a normal vasoconstriction after local cooling added on vessels with organic changes seen in patients with thromboangiitis obliterans.

The CCT-WA technique could eliminate vasospasm and thereby discriminate between pathological values due to vasospasm or due to organic obstructive changes. Measurements of the skin circulation as a complement, along with recordings of FSBP, appears to be a necessary approach to discriminate between different forms of Raynaud's phenomenon and to secure the diagnosis.

The results indicate that hypothenar hammer syndrome is a pure organic disease. The ability of the method to select these patients is a great advantage since these patients can be operated and released from their symptoms. Primary Raynaud's phenomenon seems to be a pure vasospastic disease, with basal vasospasm induced by the sympathetic nervous system and local cold induced vasospasm caused by other factors, probably local. This increased local cold sensitivity has earlier been proposed (45) but the proportions between sympathetically induced vasospasm and other vasospastic factors have never been objectively measured. TVD and scleroderma seem to be mixed organic and vasospastic diseases, wherein the vasospastic component is at least partly induced by the sympathetic nervous system. Porter, who is one of the most prominent authorities in this field concluded together with Blunt as late as in 1981 (14) that no test can yet accurately distinguish between either normal control subjects and patients with Raynaud's phenomenon or between the various causes of this condition. With the development of the CCT-WA method based on the CCT technique we hope to have contributed to the solution of this diagnostic dilemma.

10.4. Angiographic analysis (paper V)

When the suspicion of organic lesions is based purely on the history and the physical examination angiography can only confirm the diagnosis in approximately 50 %. If the diagnosis of organic lesions is based on an additional CCT-WA test the accuracy increases to 90 %.

By the transfemoral approach in the angiographic examination instead of direct puncture of the brachial artery we were able to diagnose 7 proximal organic changes. Angiographies performed by meglumine metrizoate caused severe pain in the arm. The shift to metrizamide effectively eliminated pain. In the initial angiogram the mean CWT, i.e., the time for the contrast medium to pass from the tip of the catheter to the radial artery at the wrist level was 10 seconds (range 2-20 seconds).

10.4.1. Comparison between different vasodilating procedures

See Table II.

1) Blockade of the brachial plexus was inconvenient for the patient, time-consuming but highly effective as evaluated from the CWTs which were less than 4 sec with distal filling of the plexa in 8 of the 17 patients. Release of vasospasm was also achieved, as judged from local cooling which did not change the angiographic picture. The other 9 patients did not get complete release of vasospasm after this blockade but this failure was due to technical problems, as incomplete blockade, and probably not related to pathophysiological phenomena.

2) Intraarterial injection of phentolamine produced a pronounced decrease in the mean CWT to 4 seconds (from the original 11 seconds in this group). Two patients reacted with only slight vasodilation after this treatment, but after an additional blockade of the brachial plexus was performed no cold-induced vasospasm was achieved. This indicates that vasoconstriction in some patient groups is not sympathetically induced but induced by other neurogenic mechanisms which are mediated via the brachial plexus (5-HT fibres ?).

3) Body warming markedly decreased the mean CWT to 5 seconds. Some vasospasm was, however, registered after local cooling.

4) Body warming in combination with i.a. phentolamine injection produced the shortest mean CWT without any vasospasm after local cold provocation.

5) Body warming combined with orally intake of alcohol was not superior to the above-mentioned treatments.

6) Reserpine in combination with other vasodilating procedures did not result in any angiographically visible vasodilation until 40 minutes after the drug was injected. This therapeutic combination has the

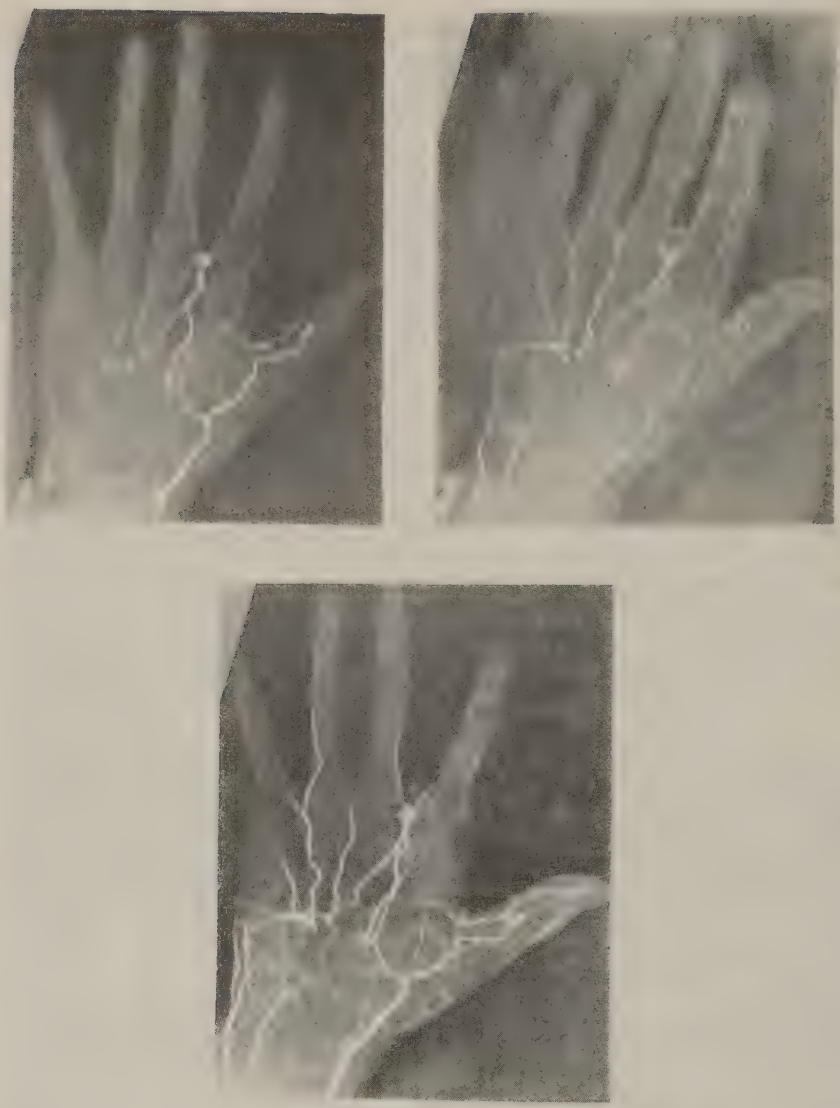

Fig 7. Angiograms before vasodilation (A), after body warming (B) and after injection of 4 mg of phentolamine (C).

disadvantage of being as time-consuming as blockade of the brachial plexus, with increasing risks for angiographic complications.

Angiography of the hand has previously been reported valuable to distinguish between some of the more than 30 different diseases or lesions causing the symptom called Raynaud's phenomenon (19, 54, 59, 78, 90). Angiography is, however, presently suggested to have its greatest value visualization of such organic lesions which can be surgically removed (49, 84). The frequent use of brachial artery catheterization together with various types of trauma caused by, for example, vibrating tools have increased the incidence of complications such as emboli, stenoses or occlusions causing a Raynaud's phenomenon and this has created a new demand for angiography of the hand. The development of microvascular techniques has also made more peripheral areas available for vascular reconstructions (62). Vasospasm is often present together with organic obstructive changes in patients with Raynaud's phenomenon. An optimal angiographic technique should be able to eliminate this vasospasm independent of its etiology in order to visualize organic obstructive changes. The investigation should also be as short lasting and as comfortable as possible for the patient and without side effects of the contrast media or the vasodilating procedures.

The importance of using two vasodilating treatments in combination to achieve a maximal vasodilating effect has been pointed out for other methods previously (75, 86) and was confirmed in the present angiographic study.

10.4.2. Concluding remarks (paper V)

Based on these results we recommend that selection of patients for angiography is made from the results obtained by the non-invasive CCT-WA technique which has a high accuracy. The angiographic investigation which should lead to a visualization of organic changes in areas available for vascular surgery should be performed after elimination of vasospasm which is usually simultaneously present. This inhibition of vasospasm is best achieved by body warming in combination with intraarterial injection of 4 mg phentolamine which implies a short-lasting investigation without pharmacological side effects. An additional blockade of the brachial plexus eliminates the rare cases of vasospasm which are not affected by phentolamine injection (Fig 7).

10.5. Reserpine treatment (Paper VI)

Besides surgical sympathectomy, reserpine has probably been the most widely used therapy for different forms of Raynaud's phenomenon (1, 51). Clinical improvement up to 6 months after a few days therapy has been reported (51). In paper III we offer the rational counterpart to the therapeutic use of this NA-depleting agent, and in paper V the vasodilating effect of reserpine is judged by angiography.

We used the CCT technique in this study in order to objectively measure the effect of one intraarterial injection of 0.5 mg reserpine in patients with primary Raynaud's phenomenon. Six patients were subjected to repeated CCT measurements and the results were compared to those obtained with a clinical scoring of their symptoms.

All 6 patients had a complete closure of the arteries after local cooling. The temperatures causing this closing phenomenon ranged from 10°C to 22°C. This range indicated that not all of these patients had a Raynaud's disease according to the criteria stated by Allen and Brown (5).

Immediately after the reserpine injection the hand and forearm became pale and painful as a result of the immediate release of NA from the sympathetic nerve endings. Two hours after the injection the forearm and hand were red. Two patients had a clinical effect of the drug only for two days. Retrospectively these were probably the two patients who had the most clearcut form of primary Raynaud's phenomenon. The other 4 patients showed an effect for 6-8 days, with the exception of one patient who had only minor complaints of recurrency after 4 weeks. This patient's main clinical problem were before the injection ulcers and tender fingertips and the long-lasting improvement was probably due to the healing of her ulcers. The pronounced vasodilation seen in the skin vessels, would make this drug beneficial for healing of digital ulcers independent of their etiology. The long-lasting effect of one single injection could also make the drug useful to eliminate the vasospasm often achieved during surgery of the vessels in the upper extremity.

10.5.1. Concluding remarks (paper VI)

An intraarterial injection of 0.5 mg reserpine eliminated closure of the arteries induced by local cooling or decreased significantly the temperature level for this closure in patients with primary Raynaud's phenomenon. The maximal effect of the drug was achieved within 48 hours after injection and the effect remained not more than 8 days. The more long-lasting improvement seen in one patients is probably due to the healing of her ulcers. The CCT method can successfully be used for an objective evaluation of provided therapy.

GENERAL CONCLUSIONS

The digital circulation was evaluated in normal healthy subjects by a finger pletysmographic method before and after local cold provocation at rest and with the measurements repeated after body warming and after body warming in combination with alcohol (the CCT-WA method). No differences related to age or sex were found. Among healthy subjects local cooling induced signs of vasoconstriction only in tobacco smokers. This cold-induced vasoconstriction was not affected by inhibition of the sympathetic nervous system achieved through body warming, but it was eliminated after combining body warming with oral ethylalcohol intake (30 ml of a 40 % solution). The observation suggests a special local vasodilatating mechanism for alcohol.

Patients with Raynaud's phenomenon, except those with neurological disorders and skin trauma, reacted compared to healthy subjects with an increased vasoconstriction after local cooling. The degree of this vasoconstriction was not correlated to the degree of prevailing circulatory impairment as reflected in the level of the finger blood pressure before local cooling. This may indicate a truly increased cold sensitivity in certain patient groups.

The CCT-WA method can efficiently discriminate between a reduced circulation due to vasospasm or to organic obstructive changes in the vascular bed. Simultaneous registration of skin temperature increases further the efficiency of this discrimination (see Table III). These possibilities for discrimination have considerable etiologic and therapeutic implications.

Isolated organic occlusions accessible to surgery can be diagnosed by the CCT-WA method and the indication for angiography can thereby be reduced. Angiography should instead be restricted for a preoperative descriptive examination of the proposed vascular lesion. The examination is preferably performed after body warming in combination with intraarterial injection of 4 mg of the α-receptor antagonist, phentolamine. In comparison with this antagonist, the NA-depleting agent, reserpine, was found to result in a more long-lasting release of local cold-induced vasoconstriction in the hand.

The contractile response to adrenergic agonists and 5-HT was investigated in isolated hand vessels. The quantitative pharmacologic analysis showed the existence of α-adrenergic and 5-HT receptors. This provides a rational pharmacologic counterpart to the therapeutic use of reserpine, and α-receptor blocking agents and 5-HT antagonists in Raynaud's phenomenon. The CCT method makes possible objective measurements of the effect of a given therapy.

Diagnosis	Skin temp.	FSBP/ ABP	FSBP 10°/ FSBP	Rewarming rate	Skin temp.	FSBP/ ABP	FSBP 10°/ FSBP	Rewarming rate	Skin temp.	FSBP/ ABP	FSBP 10°/ FSBP	Rewarming rate
Traumatic vasospastic disease	↓	↓	↓↓	↓	N	↓	↓↓ ↗	↓ ↗	N	↓	↓ ↗	↓ ↗
Hypothenar hammer syndrome	N	↓↓	N	↓	N	↓↓	N	↓ ↗	N	↓↓	N	N
Scleroderma	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓ ↗	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓
Primary Raynaud's phenomenon	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓↓	↓ ↗	↓ ↗	(N)	↓ ↗
Skin trauma	↓↓	N	N	↓↓	↓↓	N	N	↓↓	↓↓	N	N	↓↓
Carpal tunnel syndrome	N	N	N	↓	↓	N	N	↓	N	N	N	↓ ↗

N = no significant difference from control values

↓ = lower than control values

↓↓ = markedly lower than control values

↗ = increase compared to preceding procedure

FUTURE ASPECTS

It seems to be no overstatement to say that the major obstacle when dealing with patients suffering from "white cold hands" is the lack of explorative diagnostic techniques defining the underlying mechanisms in a sufficiently descriptive manner to allow for rational pharmacological or surgical therapies. Clinical physiological approaches have been presented in this study providing descriptive diagnoses that will discriminate between the various pathophysiologic mechanisms underlying the Raynaud's phenomenon, which has been a disease of theories ever since it was first described more than a century ago. This has minimized the need for angiography, but still allows for selection of patients requiring surgical treatment of their organic obstructive changes. The therapeutical trials in patients with Raynaud's phenomenon due to vasospasm have been based on a much too oversimplified opinion about a sympathetic component in the vaso-constriction.

The pharmacological in vitro studies have defined the existence of sympathetic nerves and adrenergic as well as serotonergic vaso-constrictor receptors both in hand arteries and veins. From this knowledge a comparison will be made on any quantitative changes in these parameters that may appear in patients with Raynaud's phenomenon. Such a study will hopefully enable a more rational selection of adequate pharmacological therapy to relieve the vasospasm.

In the analysis of vasospastic phenomena (i.e. without organic obstructive changes) it was found that cold-induced vasoconstriction among healthy individuals only occurred in smokers. Interestingly enough this vasoconstriction could be blocked by ethylalchol ingestion. This hitherto unknown mechanism of interaction between smoking and alcohol, not necessarily engaging the sympathetic nervous system, is apparently of great socio-medical interest and will be further investigated.

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RAYNAUD'S PHENOMENON IN ARTERIAL OBSTRUCTIVE DISEASE OF THE HAND DEMONSTRATED BY LOCALLY PROVOKED COOLING

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Abstract. Finger systolic blood pressure (FSP) was measured by cuff technique before and after local cooling in three groups of patients (Raynaud's disease (7), subclavian stenoses (5), thrombo-angiitis obliterans (15)), and in 15 normals. The response to finger cooling registered as a decrease in FSP indicates an increase of digital arterial tone. In all three groups, digital arterial tone increased more than in normals during finger cooling. Patients with Raynaud's disease showed a pathological increase in arterial tone at 23.5°C with closure of the digital arteries at a mean temperature of 18.5°C. The temperature eliciting these phenomena in patients with thrombo-angiitis obliterans was about 7°C lower (16.5 and 11.0°C, respectively). Accordingly, cold sensitivity and Raynaud's phenomena in the two groups may have a different pathophysiological mechanism, namely a pathological arterial tone in Raynaud's disease vs. a normal arterial tone in obliterative diseases acting on a narrow vessel.

Raynaud's phenomenon, as defined by Hutchinson (1893), can be a symptom in a variety of diseases, among which arterial obstructive diseases on proximal or acral parts of the upper extremity and traumatic vasospatic disease are the most frequent (Coffman & Davies, 1975). These diseases may be suspected when trophical changes on fingers or toes are present and finger systolic blood pressure (FSP) is below normal. In our laboratory, an indirect method for measurement of blood pressure on the most acral parts of the extremities has been developed (Hirai, Nielsen & Lassen, 1977). In such cases, blood pressure measurement on the finger may be valuable in forecasting the prognosis of the disease (Holstein, Krähenbühl & Lassen, 1975). Obstructions or stenoses in one or more digital arteries may, however, prevail with normal FSP.

Possibilities exist to sensitize blood pressure recording by increasing blood flow through stenotic vessels, as for instance by measurements during reactive hyperæmia or heat dilatation (Carter, 1975). Many patients claim of cold fingers or Raynaud's phenomenon, and another possibility would be to measure the response in FSP to local cooling. Narrowing of the arterial lumen during cold-induced increase in vascular tone may cause a pathological decrease in FSP in arterial obliterations. The present study deals with responses to local cooling of the digital arteries by a recently developed provocation test (Nielsen & Lassen, 1977) in different groups of arterial obstructive diseases on the upper extremity, i.e. thrombo-angiitis obliterans and arteriographically verified arterial obliterations in cases of subclavian stenoses or of Raynaud's disease.

MATERIAL AND METHODS

The material consists of: 1) seven patients with Raynaud's disease with arterial stenoses or occlusions in one or more finger arteries at magnification hand arteriography (Porter et al., 1975). 2) five patients with subclavian stenoses found by stethoscopy and arteriography performed for acutely developing Raynaud's phenomenon or permanent cyanoses of one or more fingers with trophical changes. 3) 15 patients with thrombo-angiitis obliterans as diagnosed from two or more clinical criteria (Juergens, 1972): (*a*) history of gangrene of fingers or toes, (*b*) abnormally decreased toe or finger systolic blood pressures at normal skin temperature, eventually with abnormal pulses in pedal or hand arteries, (*c*) migrating phlebitis. All the patients were referred for investigation from two departments of vascular surgery and they represent therefore rather severe cases of the diseases. Data on the patients appear in Table I, showing the characteristic sex distribu-

Table I. *Clinical data on patients with primary Raynaud's disease, subclavian stenoses and thrombo-angiitis obliterans*

Case no.	Sex	Age	Duration (years)	Phlebitis	Gangrene		Foot claudication	Pulse absent		Finger systolic pressure decrease
					Finger	Toe		radial	ulnar	
Raynaud's disease										
1	f	42	22							+
2	f	36	16							
3	f	33	8.5							+
4	f	33	13							+
5	f	41	21						+	+
6	f	37	17		+					+
7	f	27	5			+			+	
$\bar{X}$ (S.D.)		35 (5)	13 (8)							
Subclavian stenosis										
8	m	48	0.5						+	+
9	m	45	0.5							
10	f	74	0.5						+	
11	m	62	10							+
12	f	72	0.25							
$\bar{X}$ (S.D.)		60 (13)	2 (4)							
Thrombo-angiitis obliterans										
13	m	57	9	+	+	+	+			+
14	m	49	7	+		+	+		+	
15	m	41	24	+		+	+			
16	m	51	8	+	+	+			+	+
17	m	41	9	+	+	+	+		+	+
18	m	47	5	+			+			
19	m	56	3	+		+	+		+	+
20	f	40	10		+		+	+		+
21	m	51	31	+		+	+		+	+
22	f	39	8			+	+		+	+
23	m	41	2			+	+			+
24	m	45	18			+	+			+
25	m	57	13	+		+	+		+	+
26	m	48	20			+				+
27	m	30	10			+		+		
$\bar{X}$ (S.D.)		46 (8)	11 (8)							

tion. The relatively high mean age in the group of patients with thrombo-angiitis is associated with the long duration of the disease, and most cases were primarily diagnosed for symptoms in the feet. All the patients claimed cold sensitivity of fingers and eventually toes, but not necessarily of Raynaud's phenomenon.

Patients were examined in supine position at room temperature (23°–25°C), fully dressed after half an hour's rest. Coffee and smoking was avoided for three hours prior to the examination, no precautions were taken as to diet. Blood pressure was measured on both upper arms, using a 12×28 cm rubber cuff with careful detection of particularly the systolic signal in the antecubital fossa either by stethoscopy or ultrasound (Parks Electronics, model 803). Stethoscopy was carried out over the vessels at the neck. Finger systolic pressure (FSP) was measured on all five fingers with an indirect method (Hirai, Nielsen & Lassen, 1977), using a plastic cuff of 2.5×10 cm on the midphalanx of the four ulnar fingers and on the proximal phalanx of the thumb with mercury-in-rubber strain gauge for volume detection on the pulp. Signals from the gauges were recorded on a plethysmograph (Medimatic, model SP2, Copenhagen).

In one or more fingers, FSP was recorded during local cooling of the midphalanx by gradually decreasing temperature (Nielsen & Lassen, 1977). With finger blood flow stopped by a torniquet on the proximal phalanx, cooling was performed for 5 min with a specially devised plastic cuff, which also allowed measurement of FSP.

Fifteen normal volunteers (members of the laboratory staff and patients with minor, non-vascular diseases) served as controls. They underwent the same investigative procedure used in the evaluation of the response to provoked cooling in the three groups of patients. Statistical evaluation was performed as parametric statistics and testing was made with Student's *t*-test.

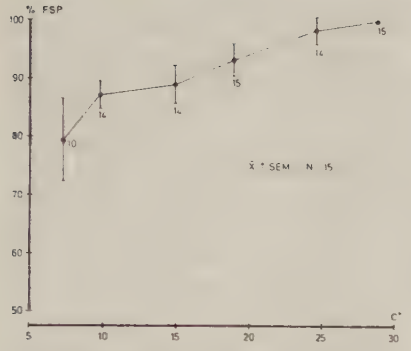

Fig. 1. Percentage decrease in finger systolic pressure (FSP) measured on midphalanx II in 15 normal volunteers during gradual decrease in temperature.

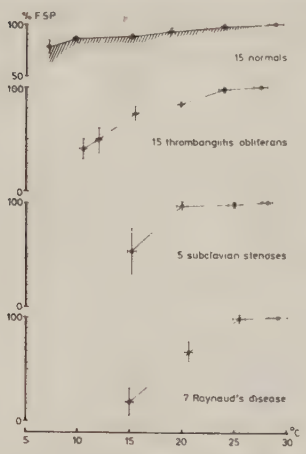

Fig. 3. Percentage change in finger systolic pressure in normals and the three groups of patients during gradual lowering of finger temperature. The values given are mean and standard error of mean.

RESULTS

The percentage decrease in FSP during local finger cooling in 15 normal subjects is given in Fig. 1. It appears that FSP shows a gradual decrease during lowering of digital artery temperature corresponding to an increase in the arterial tone. The latter was controlled by simultaneous measurement of finger pressure in a warm finger of the same hand giving the pressure in the supplying arteries, i.e. the palmar arch and proximal digital arteries.

Fig. 2 shows a typical curve from a patient with Raynaud's disease with arterial obstruction. It is seen that FSP shows a faster decline than in normals, reaching zero pressure at 14.5°C. This indicates that the arterial tone by cooling becomes greater than the transmural distending pressure leading to closure of the digital arteries. From the curves, the following figures can be obtained: finger temperature before cooling, temperature and FSP when arterial tone is increased more than in normal subjects (Fig. 1) (later denoted threshold temperature and pressure), and temperature at which the closure takes place. These parameters are given for the groups of patients in Table II.

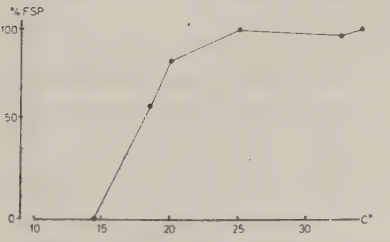

Fig. 2. Change in finger systolic pressure during local cooling of the midphalanx in a patient with Raynaud's disease.

It appears from Table II that FSP measured before cooling was significantly lower than the systolic arm blood pressure in all three groups. FSP in the patients with Raynaud's disease and thrombo-angiitis obliterans was not significantly different. Against this background, it is very interesting that both the threshold temperature and the closure temperature in patients with Raynaud's disease are significantly higher than in patients with thrombo-angiitis obliterans (closing temperature 18.5±3.6°C vs. 11.0±2.0°C). Furthermore, all but one patient in the Raynaud group showed closure. This patient had been bilaterally sympathectomized 3 years earlier. Only half of the thrombo-angiitis group showed closure during cooling, which seemed independent of the absolute finger blood pressure—some being normal and some signifi-

Table II. *Blood pressure and cold provocation in the three groups of patients*

Pat. no.	Arm BP (mmHg)	Finger systolic pressure (mmHg)	Finger temp. (°C)	Threshold temp. (°C)	Closing temp. (°C)	Threshold pressure (mmHg)
Raynaud's disease						
1	122/72	96	32	28	21.5	80
2	100/68	98	34	18.5	14.5	55
3	118/60	86	31.5	22.5	22.5	0
4	124/70	35	26.5	23.5	15	20
5	108/64	68	30	28	21	58
6	118/80	42	33	28	–	34
7	104/70	110	29	16.5	16.5	0
$\bar{X}$ (S.D.)	113 (9)/69 (6)	76 (29)	30.9 (2.6)	23.5 (4.0)	18.5 (3.6)	35 (30)
Subclavian stenosis						
8	120/70	86	25.5	17	17	0
9	128/80	125	33	10	–	88
10	158/90	140	27	24	14	122
11	150/94	115	33.5	15	–	90
12	198/108	183	26	–	–	–
$\bar{X}$ (S.D.)	151 (31)/88 (14)	129 (36)	29.0 (3.9)	16.5 (6.0)		75 (52)
Thrombo-angiitis obliterans						
13	190/80	160	33	–	–	–
14	124/80	99	32	18.5	12.5	74
15	132/86	122	33.5	15.5	–	95
16	118/70	70	29.5	12	12	56
17	130/70	50	30	20	–	38
18	122/72	117	31.5	15.5	–	90
19	140/80	63	25	20	12	50
20	148/92	48	29	19	–	35
21	120/78	63	26.5	20	11	35
22	184/110	90	22	13	13	0
23	122/72	64	26	13	7	50
24	114/70	78	24.5	15	12	62
25	154/90	103	23	19	–	80
26	138/90	90	30	11	11	0
27	142/82	120	26.5	19	–	60
$\bar{X}$ (S.D.)	139 (23)/81 (11)	89 (31)	26.1 (6.9)	16.5 (3.0)	11.0 (2.0)	51 (28)

cantly lowered (Table 2). Fig. 3 illustrates the percentage change in finger systolic pressure for normals and patients. There is a clear difference between normal and pathological response, and fingers with Raynaud's disease have a response curve skewed to higher temperature as compared to thrombo-angiitis obliterans.

DISCUSSION

Raynaud's phenomenon in its secondary form has been discussed several times (Juergens, 1972), but its pathophysiology is as yet not defined. Ashton (1962) commented that a decrease of the intra-arterial blood pressure predisposes to closure of vessels. This is the commonly held view for the appearance of the phenomenon in obliterative arterial disease. However, to provoke the typical attack with demarcated white fingers, all digital arteries in the finger should close and in many instances we found a normal finger pressure in fingers showing the typical phenomenon. The intra-arterial pressure may therefore not be the only factor involved and other mechanisms should be explored.

From a physiological point of view, a digital artery will close according to the Laplacian equation if the transmural pressure as distending force becomes smaller than the wall tension. (Gaskell, 1965.) Local hyperplasia of the intimal wall (Allen, 1937), or local stenoses (Lewis, 1938) could create a

hindrance to flow if the vessel constricts. Structural changes in the subintimal and muscular part of the artery, as for instance an increased amount of collagen tissue or muscular hyperplasia, could change the effect of a certain increase in smooth muscle tone (Coffman, 1975).

The temperature at which the digital arteries showed closure was significantly higher in Raynaud's disease than in patients with thromboangiitis obliterans. The reduction in finger systolic blood pressure was similar in the two groups and it is therefore natural to assume that, apart from organic stenoses, patients with Raynaud's disease have an increased digital cold sensitivity. Development of obliterations in severe cases of Raynaud's disease could be caused by the pathological temperature response, whatever its nature may be. The digital arterial tone was elevated at high environmental temperatures (23.5±4.0°C). Arteries in this group of patients may therefore be severed by pronounced vasospasm, as reported for ergotamine intoxication (Yater & Cahill, 1936). In less severe cases with a lower temperature threshold, the intermittency of the disease may prevent the serious trophical changes. Therefore a quantitative test of provoked cooling could possibly be used as a predictive test for diagnosing the severity of the Raynaud's disease.

The closure temperature obtained by such a test gives an impression of the frequency of the attacks of Raynaud's phenomenon at the prevailing environmental temperature. This frequency depends, however, also on the possibility for repetitive closure of all digital arteries at the same time. From records of patients with Raynaud's disease, it is indicated that some time (from minutes to hours) should elapse before the same temperature provocation leads to an attack. This could suggest that a sudden release of norepinephrine from sympathetic nerve terminals create a high vascular tone necessary for development of the attack. In patients with arterial obliterations from thrombo-embolic episodes or thrombo-angiitis obliterans, there seems to be a tendency to repetitivity of the attacks when the temperature is repeatedly lowered. This could indicate that the attack is provoked by a normal increase in vascular tone acting on an abnormal vessel (Coffman & Davies, 1975).

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A METHOD FOR THE EVALUATION OF FINGER CIRCULATION - WITH REFERENCE VALUES FOR SMOKERS AND NON-SMOKERS

Running title: Evaluation of finger circulation.

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INDEX WORDS. Finger systolic blood pressure; skin temperature; finger circulation; smoking; ethanol; body warming; vasospasm.

SUMMARY

A method for the evaluation of the finger circulation by measurements of fingertip temperature, rewarming rate and finger systolic blood pressure (FSBP) before and after local cooling is presented. Measurements were repeated after body warming and after body warming in combination with alcohol. Reference values are given for 53 healthy volunteers, 29 of which were smokers. No differences were registered between age groups or sexes.

Before body warming the mean fingertip temperature for the left hand was 31.7 ± 3.5°C (mean value ± SD; range 25-36°C) with no significant differences between smokers and non-smokers. After body warming (15 min), the mean fingertip temperature increased significantly for the whole group (33.7 ± 2.5°C) and further to 34.5 ± 1.4°C after continued body warming in combination with alcohol. Rewarming rates also rose after body warming without or with alcohol

There were no differences in FSBP:s between smokers and non-smokers, and no change after body warming and after body warming in combination with alcohol. Local cooling reduced the FSBP more in the smokers. The reduction of FSBP was more pronounced at 15°C than at 10°C. Body warming did not influence this cold-induced decrease in FSBP while body warming plus alcohol abolished the reduction in smokers. The results indicate that body warming induces a maximal fingertip temperature both in smokers and non-smokers but has no effect on FSBP in these two groups or on the cold induced decrease of FSBP registered in smokers. It is suggested that alcohol abolishes cold-induced vasospasm in smokers by another mechanism than the inhibition of sympathetic vasoconstriction caused by body warming.

INTRODUCTION

Methods for measuring finger circulation have been almost entirely worked out to evaluate objectively the symptom of white cold fingers induced by cooling or emotional stress, called Raynaud's phenomenon (Hutchkinson 1901; Coffman and Davies 1975). In 1901 Hutchkinson suggested that this phenomenon was caused either by vasospasm or organic obstructive changes. This main classification is still valid but over time it has become obvious that there are many different conditions resulting in vasospasm or organic obstructive changes (Coffman and Davies 1975; Strandness and Sumner 1975; Blunt and Porter 1981). Vasospasm can thus not only be induced by the sympathetic nervous system, as suggested by Maurice Raynaud (1862, 1874), but also by local vasoactive factors, e.g. prostaglandins and 5-hydroxytryptamine (5-HT) (cf Lewis 1929; Lewis and Pickering 1934). These factors have been reported to be inhibited by alcohol (Davis and Phillips 1970; Stuart 1979; Turlapathy, Altura and Altura 1979).

We present a method for evaluation of finger circulation in which measurements of fingertip temperature are combined with measurements of finger systolic blood pressure (FSBP), before and after local cooling to induce vasoconstriction or "vasospasm". Measurements were repeated after body warming - to inhibit sympathetic vasoconstriction (Lewis and Pickering 1931) - and after body warming in combination with alcohol - to inhibit the above mentioned local factors. We also present the results from the measurements of 53 subjects without symptoms of white cold fingers. The aim was to investigate if there is any "vasospasm" at rest or after local cooling in such subjects and if this "vasospasm" is eliminated by body warming alone or in combination with alcohol. A difference between smokers and non-smokers will be described.

MATERIAL

Fifty-three healthy volunteers without symptoms of white cold fingers or a suspicion of cardiovascular or connective tissue disorders were studied. They were divided into three age groups: $\leqq$ 30 years (10 women, 10 men), $>$ 30 $\leqq$ 45 years (10 women, 10 men), and $>$ 45 years (eight women, five men). Twenty-nine were non-smokers (12 women, 17 men) and 24 were smokers (16 women, eight men). The study was carried out from September to May. Seven participants were measured on two consecutive days and seven were measured twice during the menstruation cycle. The participants were not allowed to have tea or coffee in the morning of the investigation day or to smoke during the four hours preceding the testing. The use of drugs and oral contraceptives was noted as well as smoking habits, menstruation periods and body temperature (if this exceeded 37.5^{o}C, the investigation was postponed).

METHOD

The subjects were dressed in conventional indoor clothing and examined in a supine position at room temperature (21-22°C) after 20 min of rest. The arm blood pressure (ABP) was measured bilaterally by the auscultatory technique using a 12 x 28 cm rubber cuff.

The measurements started by registration of the fingertip temperatures of all fingers by a thermocouple (Type DU-3 Ellab, Copenhagen). Thereafter tourniquets (24 mm wide) were placed around the mid-phalanges of the third and fourth fingers of the left hand (Gundersen 1973). The tourniquet around the third finger was a specially devised plastic cuff with a double inlet for circulating water and air for the measurement of FSBP. Strain gauges (Mercury-in silastic-tube) for volume registrations were placed around the end phalanges of the two fingers (Nielsen, Bell, Lassen 1973). Changes in resistance of the gauge were recorded using a wheatstone bridge (Model SP 2 Medimatic, Copenhagen) (Fig 1).

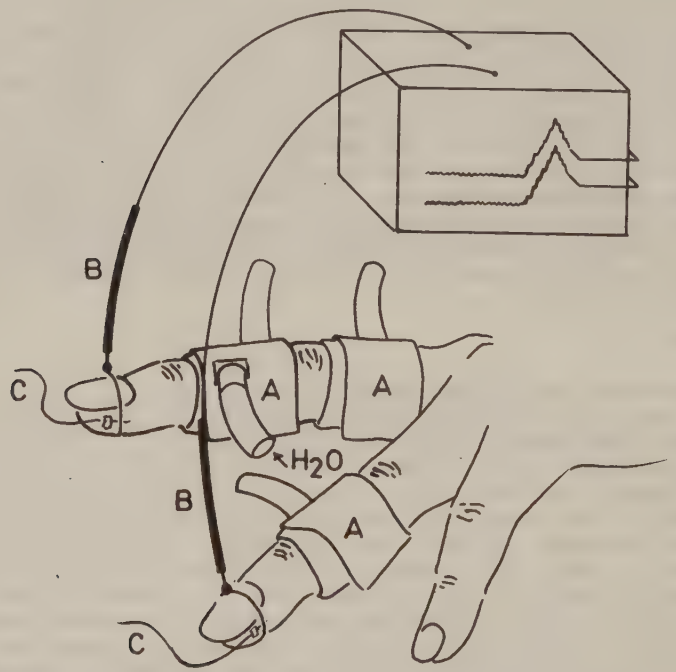

Fig 1. Schematic drawing showing the equipment with the placement of the tourniquets (A), the strain-gauges (B) and the thermocouples (C). For further explanation see Method.

The FSBP:s were measured in the two fingers whereafter the mid phalanxcuff of the third finger was perfused with water at 20°C for 5 min after arrest of the finger blood flow by a proximal tourniquet. After 5 min the mid-phalanx cuff was inflated to suprasystolic pressure and the pressure of the proximal tourniquet was released. Then the mid-phalanx cuffs were deflated by 5 mm Hg/s to zero while simultaneous volume measurements were performed over the end phalanges. This cooling procedure was also performed with perfusion of water at 15 and 10°C (Nielsen and Lassen 1977). After local cooling to 10°C the cuffs were removed and the rewarming rate was calculated from the temperature curves obtained from a thermocouple placed over the distal phalanx. Measurements were repeated after body warming for 15 min by placing an electric pad (50°C) over the abdomen and blankets over the total body except the hands. Measurements were also repeated 15 min after oral administration of 30 ml 40 % alcohol during continued body warming. A control group of six patients was measured after body warming alone for 45 min (without the addition of alcohol). The time for the investigation was one and a half hour.

The following values are presented in this paper: fingertip temperatures of the third and fourth fingers of the left hand as well as the mean values for all fingers on the left and on the right hand; the rewarming rate after local cooling to 10°C (°C/min); ABP before and after the investigation; the quotient between the initial FSBP and ABP (FSBP/ABP); the quotient between FSBP after local cooling to 15°C and 10°C, respectively, compared to FSBP before local cooling (FSBP 15°C/FSBP, FSBP 10°C/FSBP) and a cold reaction value (CRV)

$$\frac{FSBP_{t_1}\ \text{(cold finger)}}{FSBP_{t_0}\ \text{(cold finger)} - (FSBP\ t_0/\text{control finger} - FSBP\ t_1/\text{control finger})}$$

defined as the ratio of FSBP after cooling to that before cooling, corrected for variation in blood pressure measured by the change of FSBP for the control finger. A CRV of 1 means no change in FSBP after local cooling and a CRV of 0 means complete closure of the arteries after local cooling to a given temperature (CCT = critical closing temperature).

Data were analysed by classical analysis of variance. Parametric statistics were also performed with Student's t-test using levels of significance: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. The values are expressed as mean values ± standard deviation (± SD).

Analysis of variance did not indicate any differences between the age groups and sexes. The data have therefore also been analysed for the whole group. However, when smokers were compared to non-smokers there were significant differences and their data are therefore presented

separately. When comparing the data obtained from two consecutive investigations of the same subjects (n = 7) and from two investigations at different times during the menstruation cycle (n = 7), no significant differences were found except for the fingertip temperatures at rest, which were not reproducible.

RESULTS

Skin temperature for the whole group

The fingertip temperatures ranged from 25 to 36°C at rest. There were no significant differences between temperatures of the third and fourth fingers compared to the mean values for all five fingers of the left hand (Table I) and the right hand (Fig 2). The maximal temperature difference between fingers was 1.6 ± 1.0 °C. The rewarming rate after local cooling to 10°C of the third finger was 4.4 ± 1.9 °C/min.

Fig 2. The effect of body warming and body warming plus alcohol on the mean fingertip temperatures for all fingers on the left (investigated) and the right (control) hand.

Each column represents the mean ± SD. Significant differences from preceding test procedures are denoted by ●●● $p < 0.001$. Significant differences between hands are denoted by * $p < 0.05$.

Table I. The effect of body warming and body warming plus alcohol on fingertip temperatures of the left (investigated) hand with special reference to smokers and non-smokers. Mean values ± SD. Significant differences from preceding testing procedure are denoted by • $p < 0.05$, •• $p < 0.01$, ••• $p < 0.001$.

	Third finger			Fourth finger			Mean value, all fingers		
	Whole group	Non-smokers	Smokers	Whole group	Non-smokers	Smokers	Whole group	Non-smokers	Smokers
Before body warming	31.7 ±3.6	32.0 ±3.2	31.3 ±4.0	31.7 ±3.7	32.0 ±3.3	31.3 ±4.2	31.7 ±3.5	32.0 ±3.1	31.3 ±4.0
After body warming	33.7••• ±2.8	33.5• ±3.3	34.0••• ±2.2	33.5••• ±2.5	33.4• ±2.7	33.7 ±2.2	33.7••• ±2.5	33.5• ±2.8	33.9••• ±2.1
After body warming + alcohol	34.6••• ±1.7	34.2• ±2.1	35.0•• ±0.9	34.4••• ±1.5	34.2 ±1.6	34.8 ±1.2	34.5••• ±1.4	34.2• ±1.6	34.9••• ±1.1

Table II. The effect of body warming and body warming plus alcohol on FSBP measured with a water-filled cuff (third finger) and an air-filled cuff (fourth finger) with special reference to smokers and non-smokers. Mean value ± SD. Significant differences from preceding testing procedure are denoted by • $p < 0.05$.

	Third finger			Fourth finger		
	Total material	Non-smokers	Smokers	Total material	Non-smokers	Smokers
Before body warming	124.3±17.1	126.9±16.9	121.3±17.3	120.3±18.8	123.3±17.5	116.7±20.1
After body warming	124.7±16.9	128.6±14.9	120.0±18.2	116.6±17.3•	121.2±17.3	111.0±15.9•
After body warming + alcohol	127.4±17.2	131.0±16.5	123.1±17.4	118.4±17.5	121.7±16.3	114.4±18.5

After body warming the temperatures of the third and fourth fingers of the investigated hand and the mean values for all five fingers bilaterally were significantly increased (Table I). There were no differences between temperatures of the third and fourth finger compared to the mean values for all fingers of the left hand but the right hand was significantly warmer than the left one, i.e. the one in which FSBP was measured (34.3 ± 1.5°C compared to 33.7 ± 2.5°C). The conclusion can be drawn that the cuffs do not alter the skin temperatures but the whole testing procedure may lower the temperature slightly, possibly due to immobilization and elevation. The maximal temperature difference between fingers was significantly decreased to 1.1 ± 1.2°C ($p < 0.05$) after body warming and the rewarming rate after local cooling to 10°C was significantly increased to 6.3 ± 1.6°C/min ($p < 0.001$).

After body warming plus alcohol the fingertip temperatures of the third and fourth fingers of the investigated hand and the mean value for all five fingers bilaterally were significantly increased compared to after body warming alone (Table I and Fig 2). The temperature difference between the hands was not significant. The maximal temperature difference between fingers was reduced to 0.9 ± 1.2°C. The rewarming rate after local cooling of the third finger to 10°C was significantly increased, compared to after body warming without alcohol, to 7.4 ± 1.6°C/min ($p < 0.001$). Body warming alone for 45 min ($n = 6$) caused the same fingertip temperatures. The rewarming rate was slightly but insignificantly lower.

Blood pressures for the whole group

The ABP was 124 ± 15.2 mm Hg/75 ± 9.3 mm Hg before body warming. There was a tendency for a lower ABP in females under 30 but this difference was not significant. ABP after body warming alone was not measured. After body warming plus alcohol the ABP was unchanged (122 ± 15.4 mm Hg/75 ± 9.4 mm Hg).

The FSBP in the third finger was unaltered after body warming and after body warming plus alcohol (Table II). However, in the fourth finger the FSBP decreased slightly after body warming (due to the smokers - see below). There was a tendency for a lower FSBP in females under 30, but the difference to the other groups was not significant. The FSBP of the third finger measured by a water-filled cuff was significantly higher (5-7 %) than when measured by the air-filled cuff. This difference increased after body warming and after body warming plus alcohol. The quotient between FSBP/ABP was thus higher for the third than for the fourth finger (Fig 3).

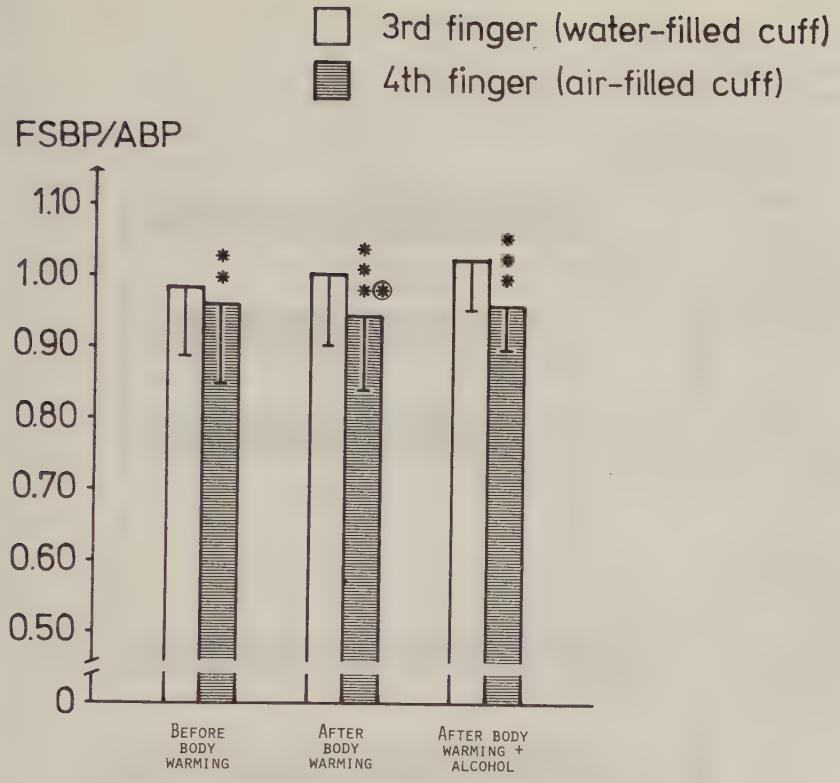

Fig 3. The quotients between FSBP and ABP (FSBP/ABP) showing the difference between FSBP measured by a water-filled cuff (third finger) and by an air-filled cuff (fourth finger). Each value represents the mean ± SD. Significant differences between fingers are denoted by ** $p < 0.01$ and *** $p < 0.001$ and significant differences from preceding test procedure ⊛ $p < 0.05$.

***

After local cooling to 10°C the FSBP decreased significantly (Fig 4). This cold-induced decrease was the same after body warming, but after body warming plus alcohol no decrease in FSBP was registered after the cooling. The CRV:s after local cooling to 15°C and 10°C, presented in Fig 5, also show the elimination of the cold-induced decrease of FSBP after body warming and alcohol. In the control group (n = 6) which was examined after body warming alone for 45 min, no changes in FSBP:s were registered compared to body warming for 15 min.

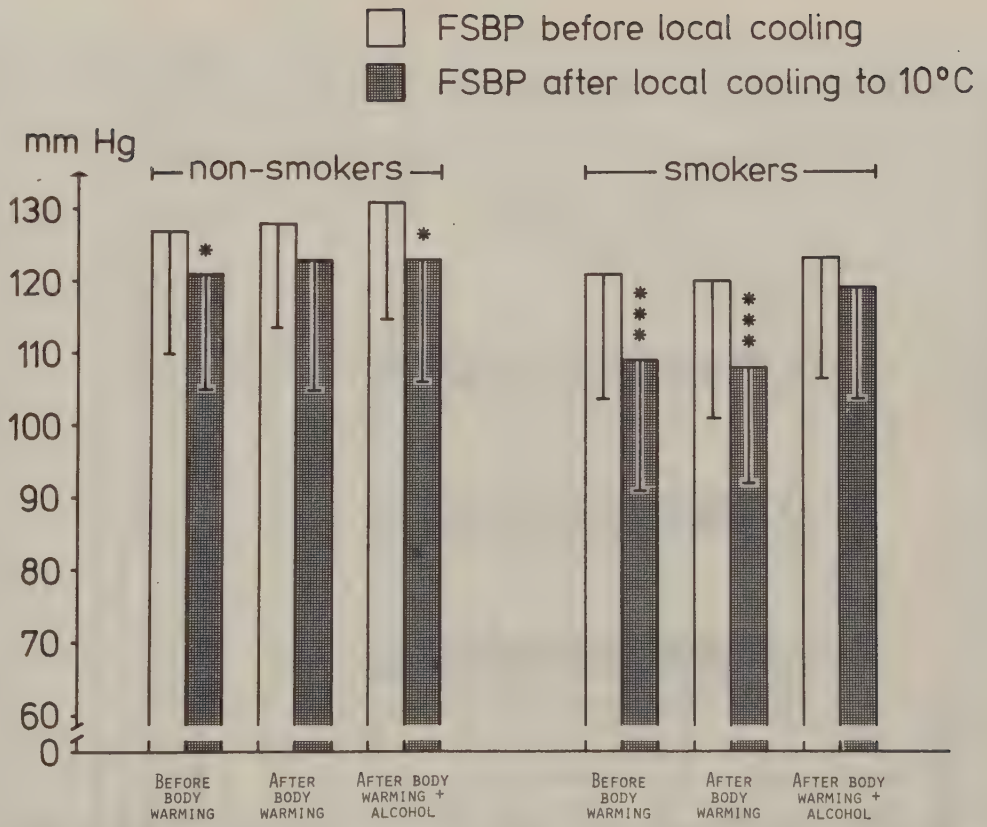

Fig 4. The effect of body warming and body warming plus alcohol on FSBP before and after local cooling to 10°C with special reference to smokers and non-smokers. Mean value ± SD. Significant differences between before and after local cooling are denoted by * $p < 0.05$ and *** $p < 0.001$.

Comparison between smokers and non-smokers

There were no significant differences in fingertip temperatures between smokers and non-smokers either before or after body warming (Table I). There were no differences between smokers and non-smokers concerning the rewarming rate after local cooling of the third finger to 10°C. The maximal temperature difference between fingers was significantly smaller after body warming for smokers when compared to non-smokers.

No significant differences in the ABP between smokers and non-smokers were noted although a tendency towards lower systolic blood pressure was observed among male smokers. The non-smoking men had an ABP of 132 mm Hg compared to 118 mm Hg for the smokers. The non-smoking as well as the smoking women had an ABP of 121 mm Hg.

The FSBP was significantly lower for the smokers (Table II). Body warming and body warming plus alcohol did not change the FSBP values except for the value in the fourth finger in smokers after body warming.

There was a significantly lower FSBP for the male smokers compared to female smokers. The non-smoking men had a FSBP (fourth finger) of 128 mm Hg compared to 112 mm Hg for the smokers. The non-smoking women had a FSBP of 116 mm Hg compared to 119 mm Hg for the smokers. This relationship remained the same after body warming and after body warming plus alcohol. The relationship was the same for the third finger. After local cooling to 15°C the FSBP decreased much more in smokers than in non-smokers (Fig 5). This decrease in FSBP was more pronounced in the smoking men than in the smoking women. The quotient FSBP 15°C/FSBP was 0.96 for non-smoking men and 0.97 for non-smoking women compared to 0.85 for smoking men and 0.90 for smoking women. After further cooling to 10°C there was still a significant decrease in FSBP for smokers compared to non-smokers (Fig 4), but the decrease was less pronounced than at 15°C (Fig 5) and the difference between the sexes was eliminated. Body warming did not markedly influence the values, but body warming plus alcohol abolished the decrease in FSBP after local cooling in smokers (Figs 4 and 5).

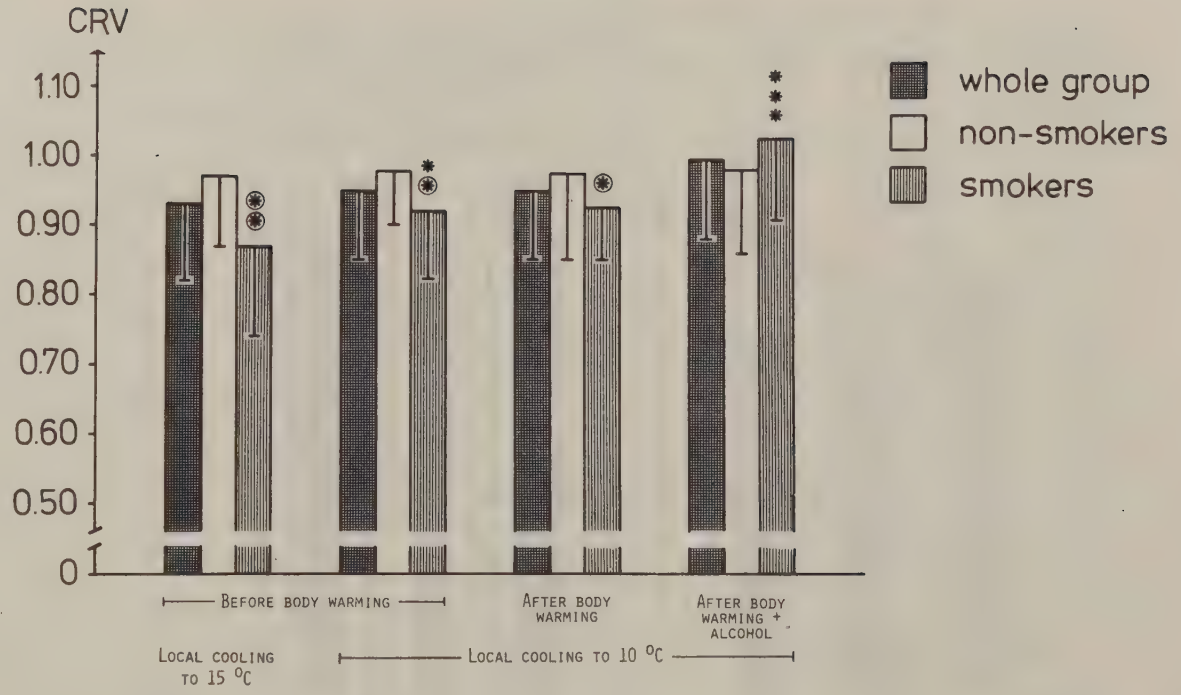

Fig 5. The quotients between FSBP after local cooling to 15°C and 10°C compared to FBSP before local cooling (when the quotients are corrected for variation in FSBP of the control finger = CRV). The cooling procedures are repeated after body warming and after body warming plus alcohol. Mean values + SD. Significant differences between smokers and non-smokers are denoted by ⊛ $p < 0.05$, ⊛⊛ $p < 0.01$. Significant differences from preceding test procedure are denoted by * $p < 0.05$, *** $p < 0.001$.

DISCUSSION

The cuff technique for the measurement of FSBP before and after local cooling was introduced by Nielsen and Lassen (1977). The aim was to find a non-invasive method which could demonstrate the possible existence of a pathological increase in arterial tone in patients with Raynaud's phenomenon. Patients with Raynaud's phenomenon showed a pathological decrease in FSBP (Nielsen et al 1978) and indeed many of the patients showed complete closure of the arteries at a particular cooling temperature (critical closing temperature = CCT). The method could not, however, differentiate between a decrease in FSBP due to pathological "vasospasm" or due to a normal cold induced constriction in arteries with organic changes. It was also obvious that the method gave normal results in some patients with clinically clear-cut Raynaud's phenomenon. This necessitated a further development of the method as described in the present paper.

The aim of including fingertip temperature and rewarming rate was to evaluate the skin circulation. We found a wide range and poor reproducibility for fingertip temperatures at rest. This is an accepted disadvantage with skin temperature measurements (Sigroth 1957).

It is also well known that skin temperatures are approximately linearly related to skin blood flow only below 32°C. In our study the mean fingertip temperature at rest was 31.7°C and thus only negligible information could be gained about the skin blood flow. It is, however, accepted that body warming gives a complete release of sympathetically induced vasoconstriction (Sigroth 1957) and that the increase in temperature - from a cooled state - after body warming is an acceptable and reproducible parameter for evaluation of the skin blood flow. Sigroth also showed that, after local cooling, the rewarming rate during body warming was a good and reproducible parameter for evaluation of skin blood flow.

We have combined this information and calculated the fingertip temperatures for all fingers bilaterally before and after body warming and after body warming plus alcohol. We have also measured the rewarming rate after local cooling to 10°C with body warming and body warming plus alcohol during the whole procedure. We found an increase in skin temperature and rewarming rate after body warming for 15 min which increased to maximal levels after continued body warming without or in combination with alcohol. This indicates that body warming for 45 min produces a maximal inhibition of the sympathetically induced vasoconstriction in the skin.

Though this release of sympathetically induced vasoconstriction by body warming is true for the skin circulation it is not necessarily true for the digital arterial circulation. A possible functional difference can have a correlate in the dual sympathetic innervation of the upper extremity (Wallin, Delius and Hagbarth 1974). They

showed that there are special sympathetic nerve fascicles which react to temperature changes and emotional stress causing vasoconstriction in the skin, while other fascicles react pulse-synchronously to sudden drop in blood pressure, and cause vasoconstriction in the skeletal muscles. To evaluate the character of the sympathetic nervous discharge to the digital arteries we measured the FSBP before and after cold-induced vasoconstriction and tried to release this vasoconstriction by body warming or by body warming plus alcohol.

The FSBP values at rest were reproducible and agree well with those presented previously (Nielsen, Bell and Lassen 1973; Thulesius, Valmin and Todoreskov 1982). To determine if a decrease in FSBP is secondary to a decrease in ABP a correlation between FSBP and ABP was used (FSBP/ABP). There is at rest a normal blood pressure drop along the arm (Nielsen, Bell and Lassen 1973). This blood pressure drop is unaffected by body warming, though there is a decrease in FSBP which is caused by a decrease in arm blood pressure (Mendlowitz and Naftchi 1959). This is in good accordance with our present findings. We have also shown that the addition of alcohol does not influence the quotient between FSBP and ABP. That the relation between FSBP and ABP is unaffected by body warming and body warming plus alcohol can probably be explained by the absence of vasoconstriction at rest. We have therefore induced vasoconstriction by local cooling.

Data have earlier been presented on a decrease of FSBP after local cooling of the finger (Nielsen and Lassen 1978). The present study can confirm these data but also show that local cooling only slightly decreased the FSBP in non-smokers; this decrease was not affected by body warming or body warming plus alcohol. In smokers, however, local cooling induced a marked decrease in FSBP. This "vasospasm" was - somewhat surprisingly - more pronounced at $15^{o}C$ than at $10^{o}C$. Hypersensibility of the finger vessels to local cooling at about $15\text{-}18^{o}C$ has been proposed previously but the material was not divided into smokers and non-smokers (Lewis and Pickering 1931; Shepherd 1963; Thulesius, Brubakk and Berlin 1981). This cold-induced vasospasm in smokers is in our present study unaffected by body warming but completely abolished after body warming in combination with alcohol.

The causes of the observed differences between smokers and non-smokers are not known. It has, however, been reported that nicotine inhibits vascular prostacyklin, but not platelet tromboxane, formation (Wennmalm and Alster 1982) and that smoking causes an increase of the aggregation of thrombocytes (Gall et al 1982). Since the subjects in our study have refrained from smoking for the last 4 hours the acute effects of nicotine can probably be ruled out.

The mechanism of action of the small dose of alcohol that we used in this study is also unknown. An effect on prostaglandin-synthesis (Stuart 1979; Cowen and Shook 1977; Kangasatio et al 1982), inhibition of 5-HT-induced aggregation of thrombocytes and

interference with the calcium metabolism inducing inability of smooth muscle contraction (Turlapaty et al 1979) have been reported. A slight effect on the central nervous system can of course not be ruled out.

We have thus shown that body warming releases the sympathetic vasoconstriction in skin vessels but does not affect the FSBP at rest and does not abolish the drop in FSBP induced by local cooling in smokers. The latter reaction can, however, be inhibited by body warming in combination with alcohol.

The present method for evaluation of finger circulation appears to be of value to elucidate the mechanism(s) behind the symptom of white cold fingers, and particularly to separate patients with Raynaud's phenomenon due to vasospasm from those with organic obstructive changes (Arneklo-Nobin, Norgren and Sjöberg, unpublished).

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ADRENERGIC AND SEROTONERGIC MECHANISMS IN HUMAN HAND ARTERIES AND VEINS STUDIED BY FLUORESCENCE HISTOCHEMISTRY AND *IN VITRO* PHARMACOLOGY

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The symptom of white cold fingers after low-temperature provocation and emotional stress is termed Raynaud's phenomenon. At present, more than 30 different diagnoses are associated with this phenomenon (Coffman and Davies 1975). The decrease in blood flow causing the symptoms are from a pathophysiological point of view due to vasospasm or organic obstructive changes. The severe vasoconstriction, "vasospasm", has been suggested to be the consequence of an overactivity of the sympathetic nervous system. Elevated levels of noradrenaline in peripheral blood has, accordingly, been reported in patients with one form of Raynaud's phenomenon (primary Raynaud's phenomenon or idiopatic vasospasm) and a defect in the degrading enzyme, monoamine oxidase, has been suggested (Peacock 1959). Therapy with α-receptor blocking agents has, however, been discouraging.

With time, the involvement of other vasoconstrictor mechanisms of more local character have been suggested. High levels of 5-hydroxy-tryptamine (5-HT) in patients with Raynaud's phenomenon were reported by Halpern et al (1960), and the therapeutic effect of 5-HT blocking agents has been successful (Jageneau et al 1982, Stranden et al 1982).

The spatial distribution of vasoconstrictor nerves have been intensely discussed (Wallin et al 1974), based on the fact that local vaso-constriction can be achieved after blockade of the peripheral nerves, but not after blockade of the brachial plexus. It has been shown that sympathetic nerve fascicles leading to the skin can be separated from fascicles supplying the skeletal muscles, with different trigger mechanisms (Wallin et al 1974). The nerves to the skin are associated with the peripheral nerve trunks, while those leading to the musculature run in close relation to the main blood vessels.

The present study was undertaken to characterize quantitatively the α-adrenergic and serotonergic receptors mediating contractile effects in isolated hand arteries and veins in vitro, utilizing material obtained during surgical operation on healthy human subjects. The aim is to provide a basis for comparison with any alterations that may occur in these properties of vessels under study from patients with Raynaud's phenomenon, and to offer a rational counterpart to the therapeutic efforts in such patients by drugs interfering with adrenergic or serotonergic vascular mechanisms.

MATERIAL AND METHODS

Material

Small segments of hand arteries and veins were obtained from a total of 60 men and women without symptoms of white cold fingers, connective tissue disorders, or cardiovascular or metabolic diseases. The age range between 20 and 60 years. The operations were performed under anesthesia of the axillary plexus by local injection of 10-15 ml of 1.5 % mepivacain chloride (Carbocain®, Astra). The preparations were removed during routine operations because of carpal tunnel syndrome and posttraumatic arthrodesis. The patients were informed in detail about the purpose of the investigation and had given their full consent to the removal of the vascular tissue.

Vascular preparations

The vessels were removed during blood-free conditions achieved by a tourniquet placed above the elbow and given a suprasystolic pressure. Short segments were dissected from small contributions of the radial artery to the thenar musculature, from the anterior interosseal artery, and from hypothenar branches of the ulnar artery, as well as from the associated veins. Attempts were made to remove vessels with a diameter less than 1 mm. Immediately after removal the vessels were transferred to an ice-cold Krebs-Ringer-glucose solution (see below for composition) and transported to the laboratory. Within 1 hour, a 5 mm long piece was mounted between two L-formed metal holders in a 50 ml manteled organ bath for recording of circular motor activity (Edvinsson et al 1974). Remaining tissue material was stored overnight in the buffer solution at $+4^{\circ}C$ in a refrigerator. The organ bath contained a modified Krebs-Ringer solution of the following composition (mM concentrations): NaCl 118; KCl 4.5; $CaCl_2 \times 2H_2O$ 2.5; $MgSO_4 \times 7H_2O$ 1.0; $NaHCO_3$ 25; KH_2PO_4 1.0; glucose 6.0. The temperature in the bath was thermostatically maintained at $+37^{\circ}C$, and the buffer solution was continuously aerated with a mixture of 95 % O_2 and 5 % CO_2, giving pH 7.40 (range 7.30-7.50). The contractile response was recorded isometrically with Grass FTO3C force-displacement transducers, and monitored on a Grass 79D polygraph.

Pieces of the excised vessels were frozen in propane-propylene at the temperature of liquid nitrogen, freeze-dried, and further processed for fluorescence microscopic demonstration of catecholamines according to the formaldehyde method of Falck and Hillarp (Björklund et al 1972). Small pieces of the vessels were in addition fixed in Bouin's solution and transverse paraffin sections of 5 μm thickness were stained in hematoxyline and eosin. These were used in order to distinguish arteries from veins, which was sometimes difficult during the blood-free operation, and for measurement of the vessel dimensions. This was used as a basis for calculating the passive load given to the vessel *in vitro* by Laplace's law, according to which wall

tension is equal to the product of the intraluminal pressure, and the radius divided by the wall thickness.

Material from hand arteries and veins was also taken for quantitative determination of the noradrenaline concentration. The tissues were homogenized in ice-cold 0.4 M perchloric acid, and the catecholamine was extracted and determined fluorimetrically according to Bertler et al (1958) and modified by Häggendal (1963).

In vitro recordings

The arterial preparations were given an initial passive load of 1 500 dynes and the veins 800 dynes, and were allowed to accomodate for 60 min. During this period, the tension decreased to 500-700 and 250-400 dynes, respectively, from which the pharmacological studies were carried out. Only tests for contractile activity were made. Agonists were added to the bath in a cumulative way (van Rossum and van den Brink 1963) to obtain full concentration-response curves, since pilot experiments had shown that fractionated application gave identical results (Fig 1).

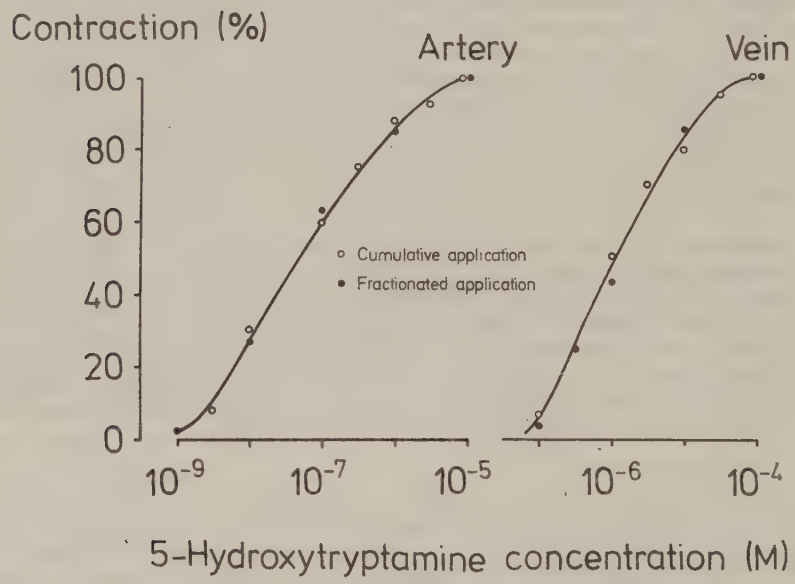

Fig 1. Representative examples of contractile response in hand artery and vein following administration of the agonist by cumulative application (stepwise in one sequence) or fractionated application (wash-out of the agonist between each single, increasing concentration). There is very close correspondence between the two modes of application.

The bath contained 10^{-6}M each of cocain and normetanephrine to block neuronal and extraneuronal uptake, respectively, of the amines (Iversen 1967), and 10^{-6}M propranolol was added to block any β-receptor mediated dilatory effects. Increasing doses of various antagonists, followed by new concentration-response curves for the agonist, were administered for the quantitative characterization of the smooth muscle receptors involved.

The following compounds were used in the tests: L-arterenol hydrochloride, L-epinephrine bitartrate, L-isoproterenol hydrochloride, 5-hydroxytryptamine (5-HT) creatinine sulfate (Sigma), phentolamine metanesulfonate (Ciba-Geigy), propranolol hydrochloride (Inderal; Scanmeda, Sweden), R49945 (Ketanserin, Janssen), cocaine hydrochloride (ACO, Sweden) and normetanephrine hydrochloride (Sigma). All concentrations are given as the salt and are expressed as the final molar concentration in the organ bath.

Treatment of data

Semilogarithmic concentration-response curves were used to estimate maximum response (E_{Am}) concentration of agonist giving half maximum response (Ed_{50}), and relative agonist potency.

According to the occupation theory of drug-receptor interaction, the formation of the receptor-agonist complex, R_A, is governed by the law of mass action (Furchgott 1972). The response of a test preparation to an agonist under steady-state conditions is considered to be a function of the concentration of the receptor-agonist complex, (R_A), times an efficacy term for that specific receptor-agonist complex. For equal responses before and after inactivation of a fraction of receptors by an irreversible type of blocking agent the following equation applies:

$$\frac{1}{(A)} = \frac{1 - q}{q} \times \frac{1}{K_A} + \frac{1}{q} \times \frac{1}{(A')}$$

where q is the amount of receptors remaining after irreversible blockade of a fraction of the total receptor population in the vessel. A plot of 1/(A) against 1/(A') should therefore give a straight line from which K_A (dissociation constant for the receptor-agonist complex) can be calculated from the relation

$$K_A = \frac{\text{slope} - 1}{\text{intercept for } 1/(A)}$$

assuming that the same concentration of receptor-agonist complex always results in the same response.

The competitive nature of the antagonism was secured in Schild plots (Arunlakshana and Schild 1959), in which the logarithm for the dose ratio minus one, plotted against the negative logarithm for the con-

centration of the antagonist, should fit a straight line with a slope of unity. Under these conditions, the intercept of the line with the axis for the antagonist concentration represents the pA_2 value, which is defined as the negative logarithm of the antagonist concentration which doubles that concentration of the agonist required to produce a given effect.

Calculations for the regression analysis and of differences between mean values (Student's t-test) were performed using a Hewlett-Packard calculator (HP41C).

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RESULTS

Fluorescence microscopic analysis of hand vessels following the formaldehyde reaction for demonstration of biogenic amines showed a well developed nerve supply of both arteries and veins. Under the optical conditions used, the nerve fibres emitted an intense green fluorescence, evidently reflecting the sympathetic neurotransmitter, noradrenaline. The sympathetic innervation was best developed in the arteries (Fig 2a), where it formed a network of fibres located in the adventitia, superimposed on the smooth muscle media layer. No fluorescent fibres penetrated into the media layer itself. The musculature in the wall of the veins was less well developed and demarcated, and here the adrenergic nerve terminals coursed in among the superficial smooth muscle cells (Fig 2b).

The results of the fluorescence histochemistry of the adrenergic nerve supply were consistent with the fluorimetric determinations of tissue noradrenaline, as shown in Table I.

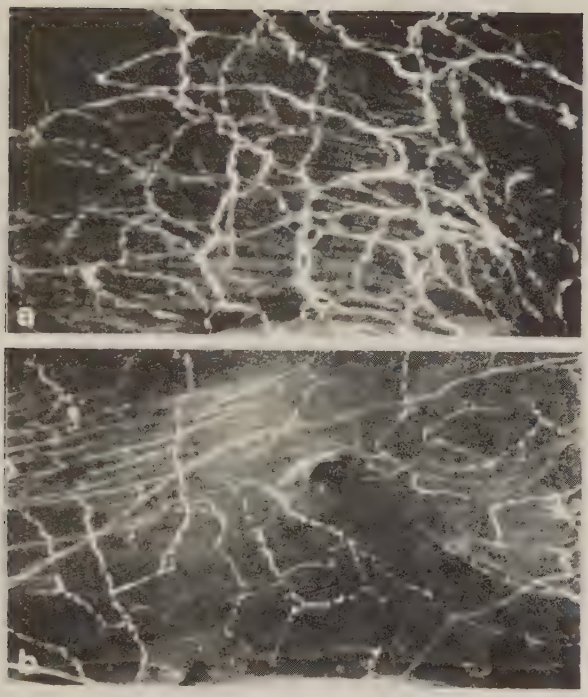


Figure 2

Fig 2. Fluorescence photomicrographs of hand artery (a) and vein (b), cut open and mounted flat on microscope slides. Formaldehyde-induced fluorescence of noradrenaline in plexuses of sympathetic nerve fibres in the vessel wall. x 150.

Measurement of the vessel dimensions (Table I) was carried out with a scale fitted in a light microscope, using transversely sectioned vascular preparations. The arteries and veins of approximately 0.7 mm in diameter had a mean wall thickness of 170 and 60 mμ, respectively. Assuming blood pressure in the test arteries to be 90 mm Hg (Folkow and Neil 1971), the transmural pressure *in vivo* would be 1 200 dynes/mm^2. According to Laplace's law, modified for blood vessels, where the wall thickness also has to be considered (Frank 1920), this corresponds to an *in vivo* tangential tension of 1 870 dynes/mm^2 for the arteries and 2 130 dynes/mm^2 for the veins, assuming an uncorrected intraluminal venous pressure *in situ* of 30 mm Hg (Folkow and Neil 1971). This was taken into consideration when giving the vessels their passive load under the *in vitro* conditions in order to avoid over-stretching of the tissue. The estimated values are summarized in Table I.

TABLE I

Various quantitative parameters of hand arteries and veins related to sympathetic innervation, dimension, and mechanical characteristics of the vessels.

Parameters	Hand artery	Hand vein
Tissue noradrenaline (ng/mg protein)	3.51 ± 0.88(6)*	3.94 ± 1.09(7)*
Estim. local blood pressure (mm Hg)	90	30
Estim. transmural pressure (dynes/mm^2)	1200	400
Approx. vessel diameter (μm)	700	700
Approx. wall thickness (μm)	170	60
Estim. tangential tension (dynes/mm^2)	1870	2130
Passive load in organ bath (dynes)	500-700	250-400
Estim. vessel tension in organ bath (dynes)	1000-1400	500-800

* Mean value ± SEM (n)

The contractile response of hand arteries and veins to the various agonists was studied by cumulative application under standardized conditions, including blockade of neuronal and extraneuronal amine uptake mechanisms, in order to allow for quantitative evaluation. The relative potency, based on the ED_{50} values (Table II), for the sympathomimetic contraction was in both arteries and veins in the order adrenaline $\geq$ noradrenaline, > isoprenaline, which is characteristic for α-receptor mediated responses. Accordingly, phentolamine shifted the dose-response curve of noradrenaline in both types of vessels towards higher agonist concentrations (Fig 3a). Schild plots gave a straight line with a slope not significantly different from unity, confirming the competitive nature of the phentolamine-induced inhibition of the contraction (Fig 3b). The quantitative values from the tests are summarized in Table II.

TABLE II

Pharmacological in vitro analysis of quantitative characteristics of adrenergic and serotonergic contractile mechanisms in hand vessels. Values are means ± SEM, together with number of tests performed in parenthesis.

Amine tested	Hand artery					Hand vein				
	ED_{50}(M)	Rel.potency	E_{Am}(dynes)	pA_2	K_A(M)	ED_{50}(M)	Rel.potency	E_{Am}(dynes)	pA_2	K_A(M)
Noradrenaline	$(1.88\pm0.51)\times10^{-7}$ (12)	1	534±121 (12)	7.57* (8)	-	$(1.07\pm0.38)\times10^{-7}$ (6)	1	516±114 (6)	7.75* (7)	-
Adrenaline	$(2.20\pm0.38)\times10^{-7}$ (12)	0.85	508±97 (12)	-	-	$(1.53\pm0.34)\times10^{-7}$ (12)	0.69	500±95 (12)	-	-
Isoprenaline	$(7.30\pm3.91)\times10^{-5}$ (5)	0.03	304±58 (5)	-	-	$(4.50\pm1.20)\times10^{-6}$ (4)	0.02	335±74 (4)	-	-
5-HT	$(2.20\pm0.27)\times10^{-7}$ (17)	0.82	518±75 (17)	9.31** (9)	8.90×10^{-7}** (12)	$(9.33\pm1.41)\times10^{-7}$ (13)	0.11	565±85 (12)	-	-

* Tested with phentolamine as antagonist.

** Tested with ketanserin as antagonist.

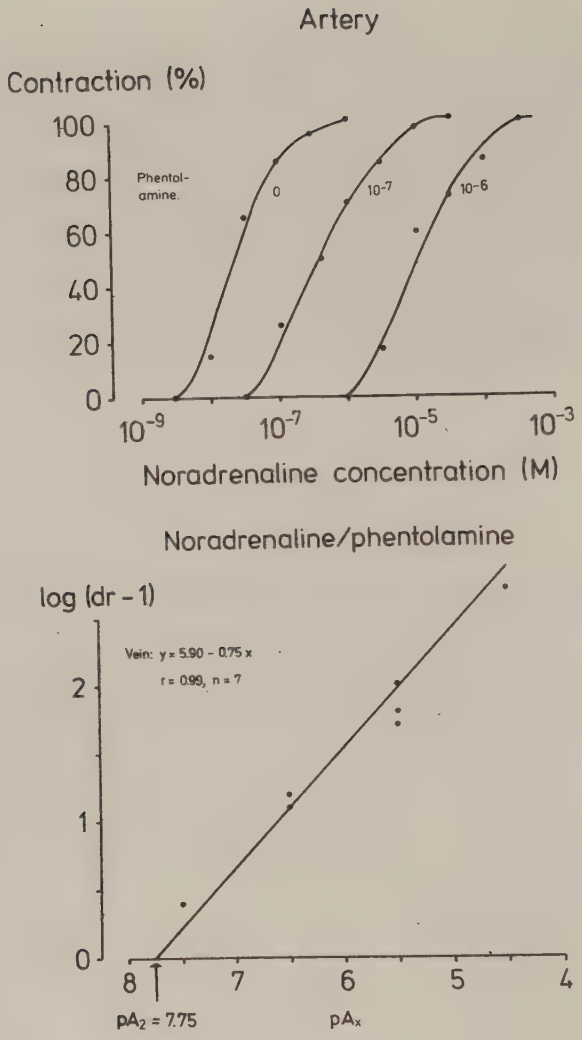


Fig 3. Interaction between the α-receptor antagonist, phentolamine, and noradrenaline. (a) Hand artery, showing parallel shift in the concentration-response curve towards higher agonist concentrations. (b) Vein, showing Schild plot based on 7 curve displacements tested on 4 vessels. The equation for the line is given, together with the correlation coefficient (r) and the number of tests (n); dr = dose ratio. The pA_2 value at the intercept of the line with the abscissa is indicated.

5-HT had an intrinsic activity on the hand arteries, as evidenced from the ED_{50} values, which was the same as that of adrenaline and noradrenaline. In the veins it was significantly lower than that of both catecholamines (Table II). The antagonist, ketanserin, counteracted the contraction induced by 5-HT in the arterial preparations by an effect involving a parallel shift towards increasing 5-HT concentrations, together with a reduction in the maximal response (Fig 5a). At the lowest antagonist concentrations (10^{-8} M) there was often a slight potentiation of the 5-HT effect. Schild plots (Fig 4) showed that the parallel shift in the arteries was the result of a true competition with the agonist according to receptor theory: the relationship between the dose ratio minus one and the negative logarithm of the ketanserin concentration showed a high degree of linearity ((r = 0.96) and the slope (-0.73) was not significantly different from -1 ($p > 0.05$).

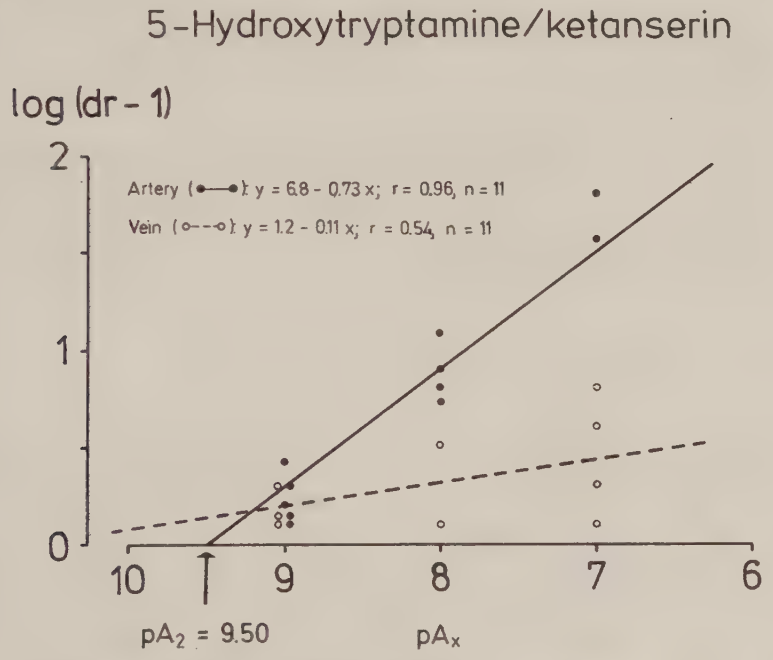
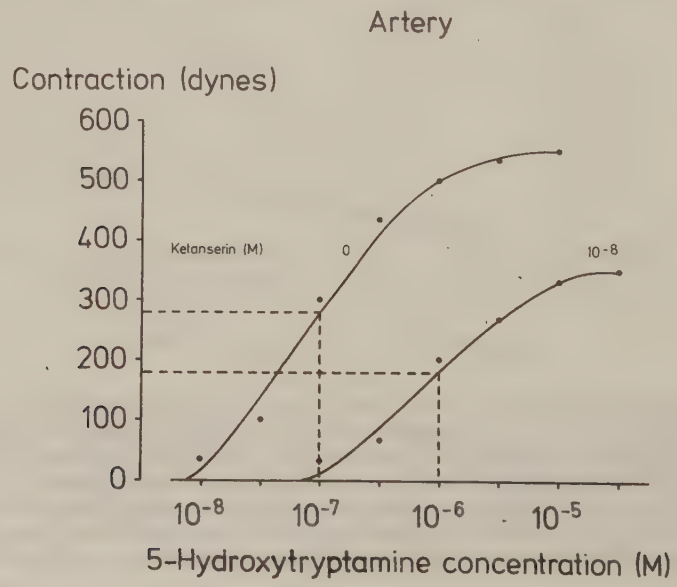


Fig 4. Schild plots from experiments on 4 arteries and 3 veins. The equation for the lines are given, together with the correlation coefficients (r) and the number (n) of tests (= dose-response curve displacements); dr = dose ratio. The pA_2 for the artery is indicated. Since the response in the veins did not fulfil the criteria for competitive antagonism, the pA_x intercept would give an erroneously high value for pA_2, which has therefore not been calculated.

The intercept between the line and the abscissa gave a pA_2 value of 9.50, demonstrating high affinity of ketanserin for the 5-HT receptor. The experiments with ketanserin on the hand veins (Fig 4) gave, in contrast to the situation in the arteries, a low degree of linearity ($r = 0.54$) in the Schild plot, and the line had a slope (-0.11) considerable lower than unity ($p < 0.001$), suggesting that other effects than true competition with 5-HT at the contractile receptor were involved.

The effect of ketanserin resulting in a reduction in the E_{Am} value remained even after repeated washing following exposure of the vessel preparation for 40 min. This observation suggested that ketanserin, besides being a competitive inhibitor in the hand arteries, also had blocked part of the 5-HT receptors irreversibly. This allowed for the calculation of the affinity (K_A) of the 5-HT for the receptor, which had a mean value of 8.90×10^{-7} M (Fig 5). Ketanserin itself caused no contraction at all of the hand vessels as tested in concentrations of 10^{-10} to 10^{-5} M. The antagonist, tested in a concentration of 10^{-7} M did not affect on the dose-response curve for noradrenaline in the hand vessels.

Also the 5-HT antagonist, methysergide, shifted the dose-response curves towards higher agonist concentrations, in combination with a reduction in the slope of the concentration-response curves. In contrast to the action of ketanserin, both components in the inhibitory response were easily eliminated by washing of the vessel preparation in the organ bath.



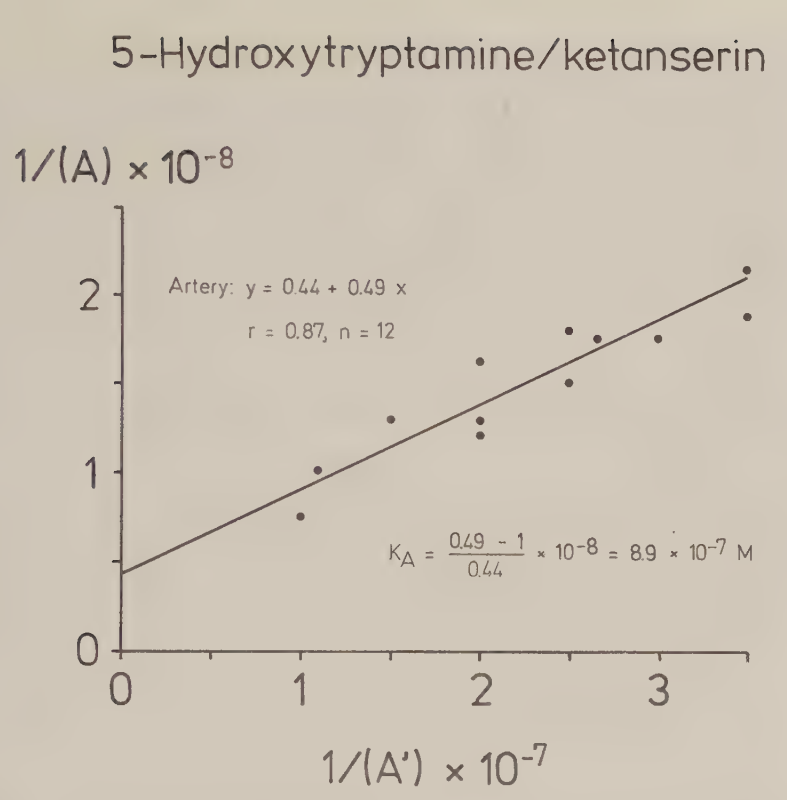


Fig 5. Interaction between 5-HT and ketanserin, which fulfilled the criteria for a competitive antagonism (cf. Fig 4) in hand arteries, including evidence for irreversible receptor blockade. (a) Example of concentration-response curves for 5-HT, shown in absolute values, before and after irreversible blockade of part of the 5-HT receptors with 10^{-8} M ketanserin (i.e. reduced E_{Am} after 40 min exposure of the vessel to the antagonist followed by thorough washing). The competitive nature of the antagonism is indicated by the parallel shift of the dose-response curve (ED_{50} values are given). (b) Calculation of the mean dissociation constant (K_A) for the complex between the serotonergic receptor and 5-HT, based on 12 tests in 4 arteries (one of which is illustrated in a). The equation for the line is given, together with the correlation coefficient (r) and number of tests (n). (A) stands for concentration of free agonist; (A') for concentration of 5-HT which, in the presence of a particular dose of ketanserin (10^{-8} M in the case of the example shown in a), gives a contractile response equal to that obtained by 5-HT in the absence of the antagonist. The equation for final calculation of K_A is presented in Material and Methods.

DISCUSSION

Both arteries and veins supplying the musculature in the proximal region of the hand were found to receive a substantial amount of innervation by adrenergic sympathetic nerves, visible with the Falck-Hillarp histofluorescence method (e.g. Björklund et al 1972). The fibres had a distribution in relation to the smooth musculature in the vessel wall which is characteristic for the autonomic nerve supply in other vascular beds (e.g. Owman 1980). The number of fluorescent sympathetic nerves agreed with the levels of tissue noradrenaline. The lower values for the arteries reflect the fact that only the muscle cells at the surface of a comparatively large mass of smooth musculature forming the medial layer are directly innervated by the sympathetic nerve terminals (Owman 1980). Further activation of deeper smooth muscle cells in the media takes place through myogenic spread of the electrical activity via low-resistance type nexus formations (Ljung 1976).

The pharmacological characteristics of the vascular response to various sympathomimetic agents, and the nature of the corresponding smooth muscle receptors, were studied under *in vitro* conditions with the vascular preparation mounted for recording of circular contractile activity. The set-up as developed in the present laboratory has been described in detail elsewhere (Nielsen and Owman 1971, Edvinsson et al 1974, Edvinsson and Owman 1974). It has been extensively applied to studies on brain vessels (Edvinsson and Owman 1977), and it has been modified and applied in several other laboratories on a variety of vascular preparations. It has a major advantage in being more sensitive than spiral-cut preparations, and it maintains the structure of the vessel wall, also to such an extent that the involvement of endothelial mediators in the motor response (Furchgott et al 1981) has been possible to explore. The vessels were given a passive load before testing, the amount being well within the estimated maximal wall tension the vessel might be exposed to under *in vivo* conditions (without correcting for forces that may act on the vessel wall from the outside *in situ*).

The presence of contractile α-adrenoceptors in hand arteries and veins was confirmed (cf. Arneklo-Nobin et al 1982). As evidenced from the pA_2 value, the α-receptor antagonist, phentolamine, had an affinity for the contractile receptor of the hand vessels that agreed well with values for corresponding receptors in other smooth muscle tissues (Furchgott 1972). Also 5-HT contracted the hand vessels, with an efficacy (E_{Am}) in the same order as the sympathomimetic response. The intrinsic activity (ED_{50}) of this amine usually resembled that of the α-receptor agonists in the arteries, and it had an intrinsic activity that was about one fifth to one tenth of that for adrenaline and noradrenaline, respectively.

The 5-HT receptor antagonist, ketanserin (van Neuten et al 1981), caused a parallel shift of the response to 5-HT in the hand arteries

towards higher agonist concentrations, and the Schild plot (Arunlakshana and Schild 1959) confirmed the competitive nature of the antagonism. Binding studies have shown that ketanserin combines specifically with a subpopulation of peripheral serotonergic receptors - occurring in vessel walls, bronchial tissue and platelets - for which the term 5-HT_2 receptors has been proposed (Leysen and Laduron 1977, Leysen et al 1978, 1981, Peroutka and Snyder 1979). The experiments have thus demonstrated the presence of 5-HT_2 receptors in human hand arteries. The mode of inhibition by ketanserin of the 5-HT induced arterial contraction allowed the calculation of the affinity of the antagonist for the serotonergic receptor (pA_2), which agreed with values obtained from previous binding studies in vitro (Leysen et al 1981).

A notable observation on the interaction between ketanserin and 5-HT in hand arteries was the poor reversibility of the 5-HT response even after thorough washing out of the antagonist following prolonged exposure. The situation resembled that reported by van Neuten et al (1981) on canine coronary arteries. This indicates that the depression of the concentration-response curves for 5-HT with increasing concentration of ketanserin might involve a component of irreversible antagonism (cf. Furchgott 1966). Advantage of this was taken to obtain a double reciprocal plot of the 5-HT response before and after partial blockade of the serotonergic receptors in hand artery preparations with ketanserin. The plot showed a high degree of linearity, and made it possible to calculate the affinity of 5-HT for its receptor (K_A). In comparison with the affinity of e.g. noradrenaline for the α-receptor, it was in the order of magnitude between that found for rabbit aortic strips (Furchgott 1972) and rat's portal vein (Johansson et al 1972) or cat's pial arteries (Edvinsson and Owman 1974).

The interaction between 5-HT and ketanserin at the level of the contractile receptor in the hand veins, in contrast to the arteries, did not fulfil the criteria for a simple bimolecular mechanism according to the law of mass action. There was no evidence that ketanserin interacted with the α-adrenoceptors in the hand vessels (cf. van Neuten et al 1981). This non-competitive antagonism, including effects of ketanserin on the veins other than those mediated by specific serotonergic receptors, resembles the situation reported by van Neuten et al (1981) on experiments with ketanserin in canine venous smooth muscle. The findings are consistent with observations in other species, showing heterogeneity in the mode the serotonergic response is antagonized in different types of vascular beds (Müller-Schweinitzer 1976, Edvinsson et al 1978, Hardebo et al 1978, Apperley et al 1980).

It has been shown that minute doses of ergotamine or dihydroergotamine sensitize vascular smooth muscle to vasoactive amines (Schönbaum et al 1976). This forms the basis for the assumption that part of the beneficial action of these compounds in migraine therapy is due to

their ability to increase the sensitivity of cranial vessels to circulating vasoconstrictor substances, thereby preventing vasodilatation. Such sensitization was presently noted with the lowest concentrations of ketanserin; it has been reported also for methysergide, as well as for pizotifen and cyproheptadine (Hardebo et al 1978). In comparison with the vascular effects of noradrenaline, histamine, acetylcholine, or potassium chloride, it was found that methysergide potentiated only the 5-HT induced conctraction, suggesting a selective mechanism for tryptaminergic agents (Hardebo et al 1978). Several mechanisms may be responsible for the phenomenon. They are probably not simply explained by the contractile effects the antagonist itself may have, but could take place either at the serotonergic receptor site or at the excitation-coupling process. Since only very low doses of the various trypaminergic agents tested were able to reveal any potentiation (Hardebo et al 1978) it is doubtful whether this sensitization has but a limited clinical importance.

The present quantitative pharmacological observations on sympathomimetic and serotonergic mechanisms in hand vessels from human subjects without symptoms of vascular disease provides a rational pharmacological counterpart to the therapeutic use of the noradrenaline-depleting agent reserpine (Abboud et al 1967, Kontos and Wasserman 1969, Willerson et al 1970) and the 5-HT antagonist, ketanserin (Jageneau et al 1982, Stranden et al 1982) in peripheral circulatory disorders, such as Raynaud's phenomenon. It shows distinctly different modes of action of ketanserin in the arteries and veins. The irreversible component in the competitive inhibition of the 5-HT induced contraction by ketanserin in the arteries may be of additional interest in the clinical application of the antagonist. Studies are in progress to evaluate pharmacological differences in the adrenergic and serotonergic mechanisms between hand vessels obtained from normal subjects and patient suffering from vascular disorders included in the Raynaud's phenomenon.

SUMMARY

Isolated hand arteries and veins from healthy human subjects were tested *in vitro* for their contractile response to adrenergic agonists and 5-HT under standardized conditions. This allowed for quantitative estimation of various receptor characteristics. The relative sympathomimetic potency suggested α-adrenergic receptors, which was continued in Schild plots following phentolamine antagonism of the response (pA_2 for artery 7.57, for vein 7.75). 5-HT contracted with an intrinsic activity approximately equal to noradrenaline and adrenaline in arteries, but only one fifth to one tenth of the catecholamine activity in veins. Ketanserin inhibited the 5-HT response in a competitive, probably also irreversible, manner in arteries (pA_2 9.31, K_A 8.90×10^{-7} M). In the veins, ketanserin counteracted the 5-HT induced contraction in a non-competitive way.

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THE DIFFERENTIATION OF RAYNAUD'S PHENOMENON BY MEASURING SKIN TEMPERATURE AND DIGITAL ARTERIAL PRESSURE, AND THEIR RESPONSES TO LOCAL COOLING AND TO VASODILATATION

Running title: Differentiation of Raynaud's phenomenon.

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ABSTRACT

The finger circulation has been evaluated in 123 patients with Raynaud's phenomenon in an attempt to differentiate between vasospasm and organic obstructive changes using a recently developed method. Measurements of fingertip temperatures were combined with measurements of finger systolic blood pressure (FSBP) before and after local cooling and repeated after body warming and after body warming plus alcohol.

In patients with vasospasm only (patients with primary Raynaud's phenomenon) the measurements showed a decrease in the FSBP/arm blood pressure (ABP) ratio and/or a cold induced decrease in FSBP which were normalized after body warming and after body warming plus alcohol. Isolated organic obstructive changes (as seen in patients with the hypothenar hammer syndrome and in some patients with traumatic vasospastic disease (TVD) gave similar changes in the FSBP/ABP ratio and in FSBP after local cooling but these changes were not influenced by body warming or alcohol. In patients with both vasospasm and organic obstructive changes (TVD and scleroderma) the proportion of each could be evaluated. Patients with the carpal tunnel syndrome and skin trauma did not show any alterations in FSBP but clear-cut changes in fingertip temperatures and rewarming rates were registered.

The present method makes possible a differentiation between vasospasm and organic changes in the vessels and also provides possibilities for objective evaluation of different therapies in Raynaud's phenomenon. The results contribute to the understanding of the pathophysiological mechanisms causing this phenomenon.

INTRODUCTION

The symptom of white cold fingers after cold provocation or emotional stress is called Raynaud's phenomenon (13). The decrease in blood flow which results in this symptom can be due to intense vasoconstriction, "vasospasm", or to organic obstructive changes (6, 13). Vasospasm in the digital vessels can be induced by an "overactivity" of the sympathetic nervous system (22, 23) or by local vasoactive processes in the digital arteries (15). This latter theory has gained support from recent findings of vasoactive properties of prostaglandins (20, 26), 5-hydroxytryptamine (5-HT) (3, 14) and calcium ions (12). The effects of these vasoactive factors are influenced by alcohol (10, 12, 26, 27).

Recently Porter concluded "that no test can yet accurately distinguish between either normal control subjects and patients with Raynaud's syndrome or the various causes of this condition" (6). In this paper we present a systematic approach to the differentiation of Raynaud's phenomenon by using a recently developed method for the evaluation of finger circulation (1). The method comprises measurements of the skin temperature, rewarming rate and finger systolic blood pressure (FSBP), before and after local cooling. Body warming and alcohol were used to release vasospasm whereafter any remaining decrease in finger circulation can be supposed to be due to organic obstructive changes. The study includes 123 patients with Raynaud's phenomenon of 6 different types.

PATIENTS

One hundred and twenty-three patients with Raynaud's phenomenon were included in the study. The phenomenon was due to traumatic vasospastic disease (TVD) in 50 patients, to hypothenar hammer syndrome in 10 patients, to scleroderma in 18 patients, to primary Raynaud's phenomenon (Raynaud's disease - idiopathic vasospasm -) in 18 patients, to the carpal tunnel syndrome (CTS) in 10 patients and to a previous skin trauma in 7 patients. The criteria for the different groups of patients as well as sex and age are presented in Table I.

All patients were subjected to a careful history taking and to relevant clinical and laboratory examinations including measurement of arm blood pressure (ABP) bilaterally, auscultation over the vessels, pulse palpation, Allen's test and determinations of hemoglobin concentration, erythrocyte sedimentation rate, antinuclear antibodies (ANA) and serum protein electrophoresis. Patients with significant differences in ABP between arms were excluded. Patients primarily included in other groups other than scleroderma were excluded if marked pathological values for the sedimentation rate, the ANA or the serum protein electrophoresis were obtained. Drug intake, contraceptive pills, smoking habits, day in the menstruation cycle and body temperature were registered. If body temperature exceeded 37.5°C the examination was postponed. The patients were not allowed to have

tea or coffee the morning of the investigation. They should have eaten and refrained from smoking at least four hours before the examination.

The investigation was performed from September to May except for five patients with primary Raynaud's phenomenon who also were examined during the summer months, because they had denied symptoms during the summer. Reference values based on examinations of 53 persons without Raynaud's phenomenon, connective tissue disorders or metabolic diseases are given in ref (1).

Table I. Data on 123 patients with Raynaud's phenomenon, and on 53 healthy controls.

	Women/ men	Mean age and range	Diagnosis based on	Arm blood pressure mm Hg
Control group women	28/-	43(18-70)	Absence of Raynaud's phenomenon, connective tissue disorders or metabolic diseases	121±3.0/74±1.2
Control group men	-/25			127±3.1/75±2.0
Traumatic Vaso-spastic Disease	-/50	44(27-65)	Occupational history	132±2.3*/83±2.2***
Hypothenar Hammer Syndrome	-/10	44(25-65)	Repeated trauma to the hypo-thenar region. Occlusion of the ulnar artery (Conn 1970)	129±9.0/80±3.6*
Scleroderma	13/5	50(24-75)	Criteria of ARA (ref)	123±3.3/71±0.9
Primary Raynaud's phenomenon	18/-	36(18-50)	Criteria defined by Allen and Brown (ref)	126±2.8/72±2.9
Skin trauma	4/3	45(30-58)	Burn injuries, chemicals and allergic reactions	138±5.2/80±3.1
Carpal tunnel syndrome	8/2	47(33-63)	Electromyography	139±6.8/83±6.2

METHOD

The patients were dressed in conventional indoor clothing and examined in the supine position at room temperature (21-22°C) after 20 min rest. Fingertip temperatures of all fingers were registered. ABP was measured. Finger systolic blood pressure (FSBP) was measured before and after local cooling of the most severely affected finger by perfusing water at 15°C or 10°C through a specially constructed finger blood pressure cuff (19). FSBP was also measured in a control finger with a normal air-filled cuff. FSBP measured by the water-filled cuff were 5-7 % higher than those obtained with the air-filled cuff (1).

After local cooling to 10°C the cuffs were removed and the rate of rewarming (°C/min) during the first 10 min was calculated from the temperature curves obtained from thermocouples placed over the distal phalanx. The rewarming rate was used, since it is less time-consuming than measuring the time of rewarming in patients with Raynaud's phenomenon (24).

Measurements were repeated after body warming for 15 min and also 15 min after orally administered 30 ml 40 % alcohol under continued body warming. The total time for the investigation was 1.5 hour.

The following variables are presented: fingertip temperatures for the most severely affected finger, the control finger and mean values for all five fingers bilaterally before and after body warming and after body warming plus alcohol; ABP; FSBP of the most severely affected finger and the control finger; the FSBP/ABP ratio and the FSBP 10°C/FSBP ratio for calculation of a cold reaction value (CRV) (defined as the FSBP 10°C/FSBP corrected for a possible change in blood pressure evaluated from FSBP:s of the control finger). A CRV of 1 means no change in FSBP after local cooling and CRV of 0 means a complete closure of the arteries after local cooling to a given temperature, i.e. critical closing temperature (CCT) (19). Finally the rewarming values after local cooling are presented.

Data were analysed by parametric statistics using Student's t-test with levels of significance * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Values are presented in the text as mean values ± SEM.

RESULTS

Reference values

Reference values based on examination of 25 healthy men and 28 healthy women are presented in Tables and Figures (see also ref 1). There were no significant differences in the values obtained for men and women.

Traumatic vasospastic disease (TVD)

The fingertip temperatures of the TVD group before body warming were significantly lower than for the control group. After body warming an increase to control group values was obtained (Table II).

Table II. Effect of body warming and body warming plus alcohol on fingertip temperatures (^{o}C) as a mean value for all five fingers of the "cuff-hand" for different groups of patients with Raynaud's phenomenon and control group. Each value represents the mean ± SEM. Significant differences from the control group are denoted by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

	Before body warming	After body warming	After body warming plus alcohol
Control group women	31.4 ± 0.7	33.4 ± 0.5	34.5 ± 0.3
Control group men	32.1 ± 0.7	34.0 ± 0.5	34.5 ± 0.3
Traumatic vasospastic disease	29.4 ± 0.8**	33.7 ± 0.3	34.1 ± 0.3
Hypothenar hammer syndrome	31.1 ± 1.0	34.1 ± 0.6	34.2 ± 0.6
Scleroderma	25.7 ± 0.9***	27.2 ± 1.2***	27.4 ± 1.2***
Primary Raynaud's phenomenon	24.1 ± 0.7***	25.4 ± 1.2***	31.7 ± 0.9**
Skin trauma	22.4 ± 0.3***	20.9 ± 0.4***	22.4 ± 0.6***
Carpal tunnel syndrome	32.3 ± 0.5	30.5 ± 0.9*	34.1 ± 0.2

The ABP - especially the diastolic pressure - of the TVD group was significantly higher than for the control group (Table I). The FSBP in both fingers were equal to those obtained for the control group before and after body warming and after body warming plus alcohol (Fig 1). Consequently the FSBP/ABP ratio was significantly lower due to the higher ABP (Fig 2).

The FSBP $10^{o}C$/FSBP ratio in the whole TVD group was significantly reduced with only slight improvement after body warming and after body warming plus alcohol. Some of the patients showed a severe vasospasm with complete closure of the arteries with CCT values varying from 10^{o} to $20^{o}C$, while others had only a slight decrease in the FSBP $10^{o}C$/FSBP ratio. Patients with a complete closure were grouped together as group A (n = 15), while the remaining patients formed group B (n = 35). The CRV (FSBP $10^{o}C$/ FSBP ratio corrected for variation in FSBP of the control finger) for groups A and B are presented in Fig 3. For group A the CRV increased after body warming and further after body warming plus alcohol. For group B, however, the CRV was completely unaltered after body warming and after body warming plus alcohol (Fig 3).

The rewarming rate of the TVD group was significantly slower than for the control group before and after body warming and after body warming plus alcohol (Fig 4).

Hypothenar hammer syndrome

Fingertip temperatures of these patients were equal to those obtained in the control group before and after body warming and after body warming plus alcohol (Table II).

The diastolic ABP of these patients was significantly higher than for the control group (Table I).

The FSBPs in both fingers of these patients were significantly reduced compared to the control group and the values were unchanged after body warming and after body warming plus alcohol. The FSBP/ABP ratio was even lower, due to the slightly higher ABP (Fig 2).

The CRV before body warming of patients with this syndrome was not different from that in the control group and the value was unaltered after body warming and after body warming plus alcohol (Fig 3).

The rewarming rate of these patients was significantly slower than for the control group before and after body warming but after body warming plus alcohol there was no difference (Fig 4).

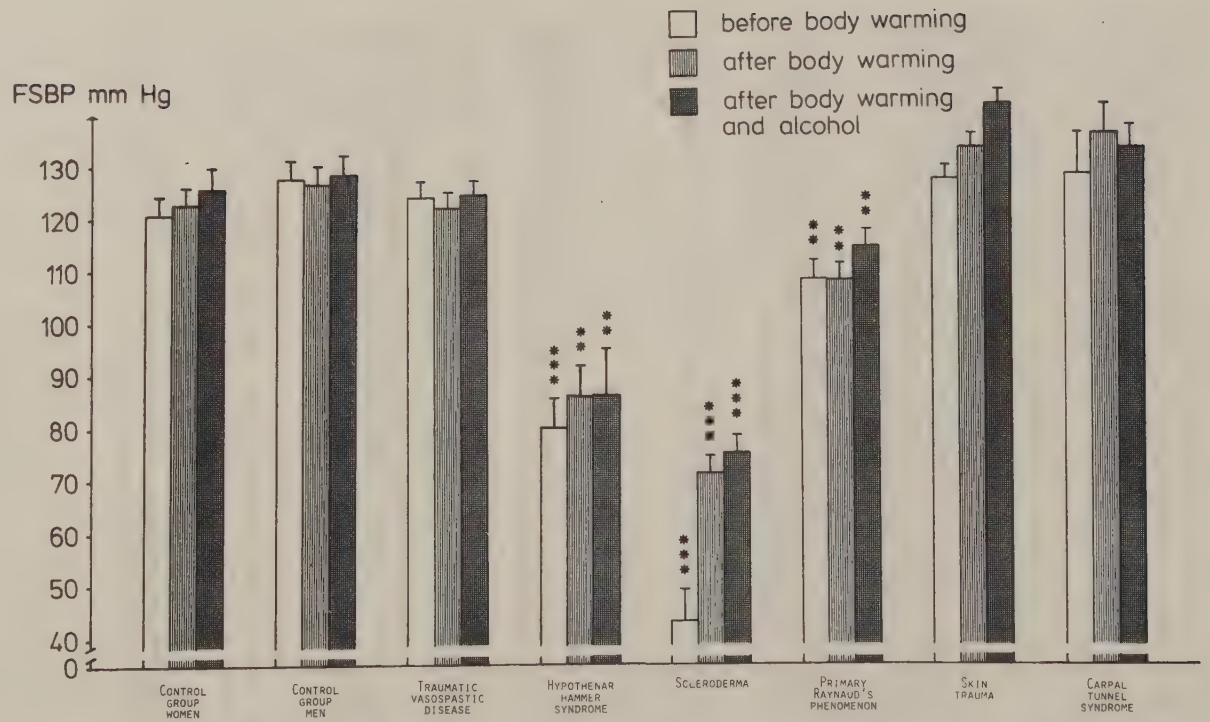

Figure 1. Effect of body warming and body warming in combination with alcohol on finger systolic blood pressure (FSBP) in mm Hg for different groups of patients with Raynaud's phenomenon and control groups.

Each value represents the mean ± SEM. Significant differences from the control group are denoted by * $p < 0.05$. ** $p < 0.01$, *** $p < 0.001$.

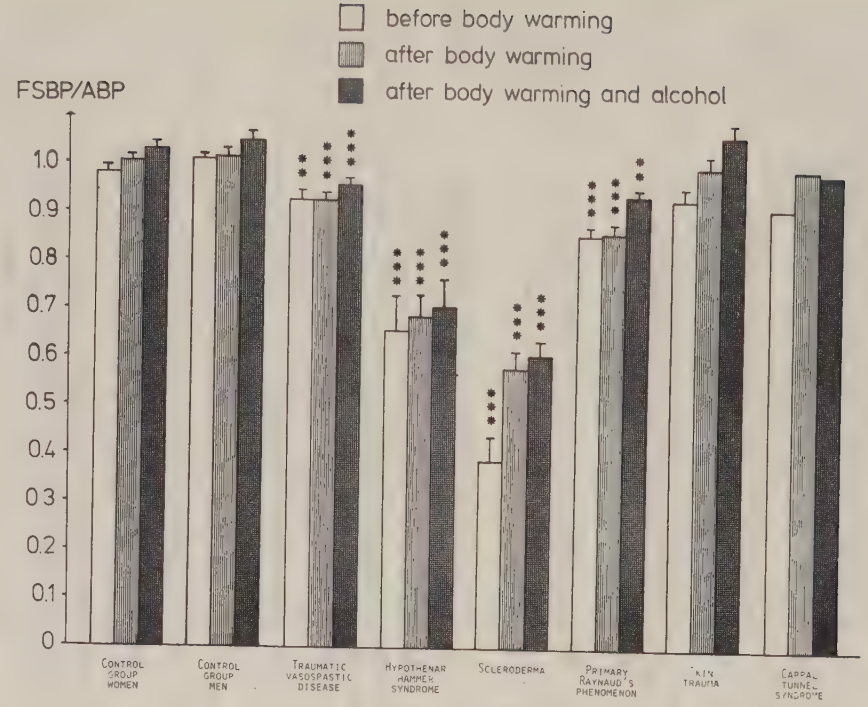

Figure 2. Effect of body warming and body warming in combination with alcohol on FSBP related to arm blood pressure (FSBP/ABP) for different groups of patients with Raynaud's phenomenon and control groups.

Each value represents the mean + SEM. Significant differences from the control group are denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Scleroderma

Fingertip temperatures before body warming were significantly lower in these patients than in the control group, with no improvement after body warming or after body warming plus alcohol (Table II).

The ABP was not significantly different from that obtained in the control group (Table I). The FSBP values before as well as after body warming and body warming plus alcohol were markedly lower compared to the control group (Fig 1). The FSBP values and the FSBP/ABP ratio were the lowest registered for any group. There was a significant increment in the FSBP/ABP after body warming but no further increase was registered after body warming plus alcohol (Fig 2).

The CRV in these patients was markedly decreased before body warming as well as after body warming and after body warming plus alcohol. An increment was, however, registered (Fig 3).

The rewarming rate in the scleroderma patients was significantly slower compared to the control group before body warming, and remained at that level after body warming and after body warming plus alcohol (Fig 4).

Primary Raynaud's phenomenon (Raynaud's disease)

The fingertip temperatures of these patients were significantly lower before body warming compared to the control group. After body warming the temperature was unaltered but increased significantly after body warming plus alcohol (Table II). The control finger - which had not been cooled - reached normal values after body warming alone indicating a long-lasting effect on the circulation after the local cooling procedure.

The ABP of these patients was not significantly different from that obtained in the control group (Table I).

The FSBP was significantly lower than for the control group before body warming. After body warming the FSBP was unaltered but an increment was observed after body warming plus alcohol (Fig 1). For the control finger, however, an increase was noted after body warming alone (for explanation: see above). Similar results were obtained for the five patients who were examined during the summer months, with the exception of fingertip temperatures which were somewhat but unsignificantly higher.

After local cooling the CRV decreased to zero. Complete closure of the arteries was obtained at almost the same temperature - 15°C to 17°C -for the whole group. After body warming the CCT was exactly the same. Body warming plus alcohol did, however, almost abolish the decrease in CRV (Fig 3). In the five patients who were also examined during the summer months the FSBP before body warming alone was not different

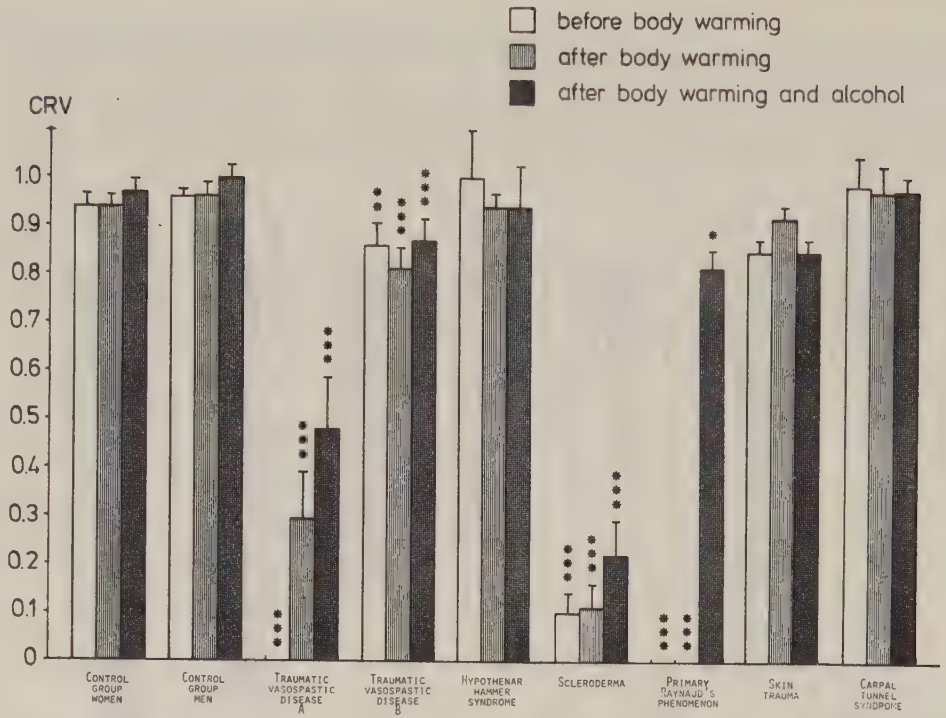

Figure 3. Effect of body warming and body warming in combination with alcohol on the CRV for different groups of patients with Raynaud's phenomenon and control groups. (CRV = $FSBP_{10^oC}/FSBP$ correlated to changes in FSBP of the control finger.)

Each value represents the mean ± SEM. Significant differences from the control group are denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

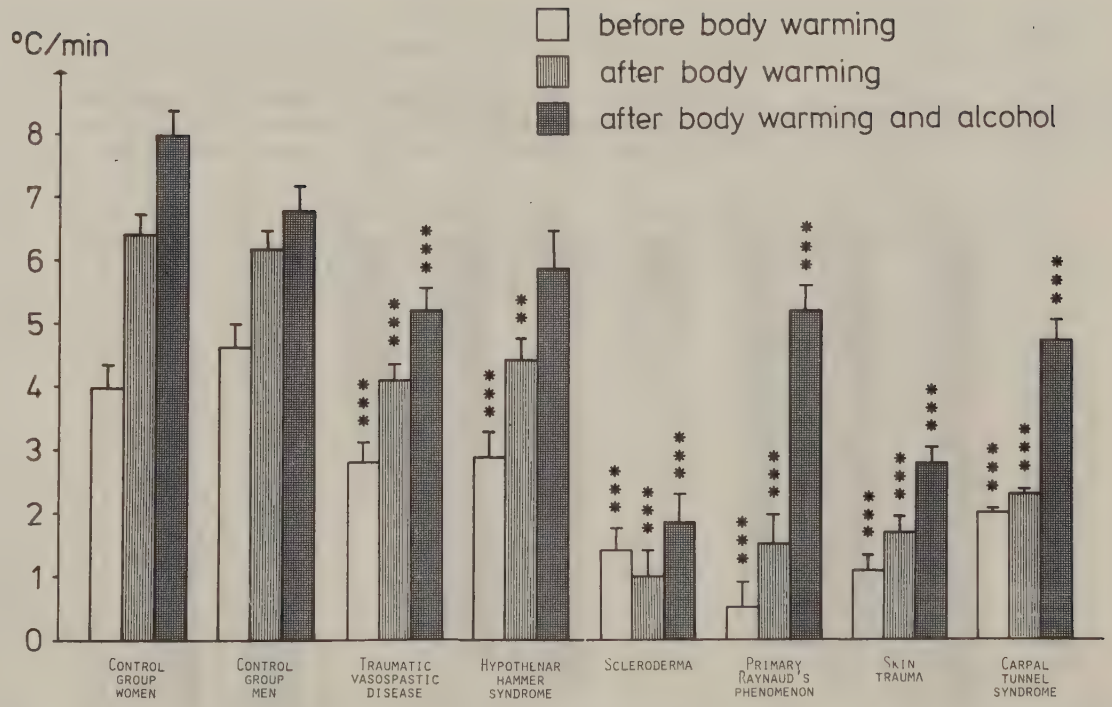

Figure 4. Effect of vasodilatating procedures performed by body warming and body warming in combination with alcohol on the rate of rewarming (°C/min) after local cooling to 10°C for different groups of patients with Raynaud's phenomenon and control groups.

Each value represents the mean ± SEM. Significant differences from the control group are denoted by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

from the one obtained in the control group.

The rewarming rate of these patients was decreased compared to the control group. After body warming the rewarming rate was increased and further increased after body warming plus alcohol (Fig 4).

Skin trauma

The fingertip temperatures were markedly lower for patients in this group compared to the control group before body warming. The temperatures decreased after body warming but after body warming plus alcohol the values were the same as before body warming (Table II). Normal values were obtained for ABP, FSBP and FSBP/ABP (Table I, Figs 1-2). The CRV before body warming was slightly decreased but normalized after body warming and after body warming plus alcohol (Fig 3).

The rewarming rate of these patients was significantly slower than for the control group and the value was unaltered after body warming but with a small increment after body warming plus alcohol (Fig 4).

These very low fingertip temperatures and the decrease in rewarming rate in combination with normal values for the FSBPs were only demonstrated in this group of patients.

Carpal tunnel syndrome

The fingertip temperatures of these patients before body warming were not significantly different from those obtained in the control group. After body warming the temperatures surprisingly decreased. After body warming plus alcohol an increase to normal values was obtained (Table II).

The ABP for this group was not significantly different from that obtained in the control group (Table I). The FSBP, FSBP/ABP ratio and the CRV were not different from the values obtained in the control group either before or after body warming or after body warming plus alcohol (Figs 1-3). The rewarming rate of these patients was significantly decreased before and after body warming while an increment was registered after body warming plus alcohol. These values were, however, still lower than the control values (Fig 4).

The combination of normal FSBP and decreased fingertip temperatures after body warming and decreased rewarming rates was only obtained in this group of patients.

DISCUSSION

Raynaud's phenomenon is at present associated with more than 30 different diseases or conditions (5, 7, 25). Discrimination between all these groups are most frequently based on clinical criteria since the exact pathophysiological mechanism behind each disease or condition is, with few exceptions (21) not known. Due to the different criteria used for the clinical classification and the multiple pathophysiological mechanisms causing the same phenomenon methods for objective assessment of a special pattern for each group have been almost impossible to work out (6, 25). The therapeutic effects have for the same reasons been hard to evaluate.

A method for the discrimination between the most important pathophysiological mechanisms behind Raynaud's phenomenon would be of great value, not least from the therapeutical point of view. The two main mechanisms are considered to be sympathetically induced vasospasm and organic obstructive changes (6, 13, 25) but additional local vasoactive mechanisms and agents causing vasoconstriction have also been suggested as have disturbances in skin innervation (3, 6, 20). In order to discriminate between vasospasm and organic obstructive changes body warming has been widely used since it has been regarded to be the most potent inhibitor of sympathetically induced vasoconstriction (16).

A methodological problem has been to provoke and evaluate the cold-induced Raynaud's phenomenon in a relevant way. An important progress was made by the introduction of a specially constructed finger-cuff with capability to circulate cold water of different temperatures and at the same time register the results by measurement of FSBP with the same cuff (19).

General cooling is a well known principle for activation of the sympathetic nervous system causing peripheral vasoconstriction. The effect of local cooling is, however, not completely known. Lewis found that vasoconstriction could be provoked by local cooling even after inhibition of the sympathetic nervous discharge by blockade of the ulnar nerve (15). We have previously shown that vasoconstriction induced by local cooling is unaltered before and after body warming while it is inhibited by body warming plus alcohol (1). Alcohol has been reported to interfere with local vasoactive mechanisms (10, 26, 27). The vasoconstriction after local cooling can therefore probably be due to other mechanisms than the sympathetically induced vasospasm achieved after general cooling.

With the above mentioned finger cuff technique a significant cold-induced decrease in FSBP was found in patients with Raynaud's phenomenon but the technique could not differentiate between a decrease in FSBP due to pathological vasospasm and a decrease due to normal cold-induced vasoconstriction in arteries with organic changes (18). The method has therefore recently been modified and at present

all measurements are performed after body warming and after body warming plus alcohol (1). A minor draw-back with the original technique was that normal results were achieved in some patients with clinically clear-cut Raynaud's phenomenon. To the present method measurements of skin temperatures have therefore been added in order to evaluate also the skin circulation.

Skin temperatures have for a long time been used for evaluation of skin circulation. Due to considerable diurnal variability (25-35°C) the fingertip temperatures have a poor reproducibility (20). After inhibition of the sympathetic discharge with body warming the temperature changes diminished thus making the evaluation of skin circulation more reproducible. Fingertip temperatures of more than 32°C after body warming are considered to indicate an absence of arterial obstructions (11). Body warming alone or in combination with alcohol gave in the present study fingertip temperatures above 32°C in the TVD group, the hypothenar hammer syndrome group, the CTS group and in the patients with primary Raynaud's phenomenon indicating absence of organic obstructive changes and probably a normal skin blood flow (11). Patients with hypothenar hammer syndrome with occlusion of the ulnar artery had in our study normal fingertip temperature already before body warming. This observation shows that temperatures above 32°C after body warming does not prove absence of arterial obstructions (11).

The very low skin temperatures before as well as after body warming and body warming plus alcohol in patients with scleroderma and skin trauma ought to indicate organic changes. These organic changes in patients with skin trauma are probably restricted to the skin, since the FSBP, FSBP/ABP and FSBP 10°C/FSBP are normal. Information gained from skin temperature measurements is therefore difficult to evaluate, even concerning the skin circulation.

The time of rewarming after local cooling is, however, reported to be a reliable and reproducible parameter for evaluation of skin blood flow (24). In this study no group of patients with Raynaud's phenomenon had a normal rewarming rate either before or after body warming. After body warming plus alcohol patients with the hypothenar hammer syndrome were the only ones who did not significantly differ from the control group. This indicates that a single proximal occlusion does not markedly affect the skin temperature or the rewarming rate.

In all patients, except for those with CTS and skin trauma, is a decrease in skin circulation parallels a decrease in digital arterial pressure. The decreased rewarming rate, the reduction in skin temperature and the normal FSBP, FSBP 10°C/FSBP, after body warming in patients with nerve compression (CTS) support the hypothesis of a separate innervation of the skin vessels. Vasodilatating fibres have for a long time been suggested (16) and the existence of vasodilatating β-adrenergic receptors in skin AV-shunts have recently

been reported (8). Nerve compression might interfere with this vasodilatating mechanism. In the skin traum group the reduction in skin temperatures and rewarming rate even after body warming and alcohol may be due to a damage of the cutaneous vessels, i.a. the AV-shunts.

Measurements of FSBP only are probably of little value unless correlated either to the ABP (FSBP/ABP) or to values obtained after local cooling (FSBP 10^{o}C/FSBP). This is verified in the group of patients with TVD where FSBP is normal but the FSBP/ABP ratio is decreased compared to the control group.

The degree of the decrease in FSBP/ABP ratio and its variation after body warming and alcohol appears of diagnostic value. A comparison was made with angiographic findings (2) in all the patients with hypothenar hammer syndrome, 15 patients with TVD and two patients with scleroderma, all of which had a FSBP/ABP ratio of 0.8 or less. A ratio of about 0.8 which normalizes after body warming is probably due to sympathetically induced vasospasm while a ratio about 0.8 without increment after body warming and body warming plus alcohol indicates an organic obstructive change. A ratio of 0.8 combined with a decreased CRV to 0 seems to indicate multiple organic stenosis or occlusions as does a ratio below 0.6. An index below 0.6 in combination with very low skin temperatures and rewarming rate without improvement after body warming and after body warming plus alcohol is in our study only found in patients with scleroderma and would indicate organic changes.

For patients with primary Raynaud's phenomenon we have shown reduced FSBP/ABP ratios not only in the winter but also in the summertime. This is in variance with the early report that this group of patients have normal FSBP (6). The normalization of FSBP already after body warming registered in the control finger indicates that the initial reduction in FSBP /ABP is due to sympathetically induced vasospasm. The reduction in FSBP after local cooling in this group of patients is, however, unaltered after body warming but almost completely abolished after body warming plus alcohol. The specific effect of alcohol in this group of patients is of great interest. Further investigations are needed to explain this phenomenon, particularly since the same pattern has been reported for cold-induced decrease in FSBP in healthy smokers (1).

The interesting observation was made that whereas patients with primary Raynaud's phenomenon all had closing temperatures within a narrow range of 15^{o}C to 17^{o}C, other patients (with TVD and scleroderma) had a much wider range (10^{o}C to 25^{o}C). The CCT achieved in patients with primary Raynaud's phenomenon is exactly the same after body warming while it often increases in patients with TVD or scleroderma. This can be explained by a possible combination of vasospasm and organic obstructive changes both in TVD and in the scleroderma groups. The organic obstructive changes in TVD have

earlier been reported to be due to a hypertrophy of the medial smooth muscle (6). It is also well known that this hypertrophy increase the tendency for a complete closure of the arteries after cold provocation. A different degree of hypertrophy in the vessels can explain why patients with TVD group B have only a slight decrease CRV, while patients with TVD group A have a decrease in CRV to zero. In this latter group the hypertrophy is probably combined with sympathetically induced vasospasm since the CRV increases after body warming and further after body warming plus alcohol, while the values are still decreased compared to those obtained for the control group. This dual pathophysiological mechanism is also obvious in the patients with scleroderma.

The present method can thus differentiate between vasospasm and organic obstructive changes. The localization and degree of the obstructive changes can also be clarified. Each group of patients with Raynaud's phenomenon seems to have its own pathophysiological pattern. It is not within the scope of the present work to explain the mechanisms behind the different patterns; for this further work is necessary. Nevertheless, it seems likely that a pathophysiological evaluation might provide a better basis for therapy than classification of the patients according to clinical criteria. Pathophysiological patterns for other groups of patients with Raynaud's phenomenon, i.e. diabetes, systemic lupus erythematodes, arteriosclerosis are under investigation in our laboratory.

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INDICATIONS FOR ANGIOGRAPHY AND ITS OPTIMAL PERFORMANCE IN PATIENTS WITH RAYNAUD'S PHENOMENON

Short title: Angiography in Raynaud's phenomenon.

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ABSTRACT

Fifty-two patients with Raynauds's phenomenon of the upper extremity were angiographed due to suspected organic stenosis or occlusions available for reconstructive vascular surgery. Different vasodilatating treatments were compared either singly or combined: blockade of the brachial plexus, intraarterial injections of phentolamine or reserpine, body warming and orally administered alcohol. Body warming in combination with 4 mg phentolamine gave maximal vasodilatation without vasospasm after local-cooling-test in patients with sympathetically induced vasospasm enabling a clear visualization of organic lesions. Close to maximal vasodilatation was obtained 40 min after blockade of the brachial plexus or after reserpine injection combined with body warming.

KEY WORDS: Hand angiography; Raynaud's phenomenon; vasodilatation.

INTRODUCTION

It has earlier been reported that angiography of the hand can be used to distinguish between some of the more than thirty different diseases or lesions which cause the symptom called Raynaud's phenomenon (11, 14, 26). Today laboratory tests for the diagnosis of, for example, autoimmune diseases (8, 20) and new non-invasive techniques for the evaluation of the peripheral circulation (12, 16, 24, 27, 29) have to a large extent replaced angiography in investigations of patients with Raynaud's phenomenon. Recently angiography has been considered to be of value only for the demonstration of a proximal, i. e., subclavian source of digital emboli, since that area was surgically correctible (6, 10, 20).

The development of the microvascular technique has made more peripheral areas available for vascular reconstructions (15). The growing frequency of brachial artery catheterization for examination of the heart (7) and trauma caused by, for example, vibrating tools, has increased the incidence of emboli, stenosis or occlusions (9) and thus created a new indication for angiography of the hand. To achieve optimal value of the angiography prior to surgery, the technique used must eliminate vasospasm when this is present simultaneously with organic obstructive changes.

In order to eliminate or control vasospasm many methods and drugs have earlier been used not only in angiography, but in all methods

measuring the peripheral circulation in the upper extremity. General anesthesia (13, 25), blockade of the stellate ganglion (4, 14) or the brachial plexus (25), α-blocking agents (4, 28), reserpine (3, 21), prostaglandins (5), oral administration of alcohol (30) and body warming (18, 22) have been used to control vasospasm induced by the sympathetic nervous system or by local factors.

The aim of the present study has been to work out the least time-consuming, most painless angiographic technique which gives an optimal visualization of the areas important for a decision about vascular reconstructive surgery in the upper extremity. Regarding the elimination of vasospasm, we have compared the effect of blockade of the brachial plexus, intraarterial injections of phentolamine or reserpine, body warming, orally administered alcohol and combinations of these treatments.

PATIENTS

Fifty-two patients with the symptom called Raynaud's phenomenon were examined with angiography of the upper extremity by the transfemoral approach. The indication for angiography was in all patients a suspicion of stenosis or occlusion of the arterial tree proximal to the basal phalanx. This suspicion was in 15 patients based on their respective histories and physical examinations including bilateral brachial blood pressure, auscultation for bruit of the vessels, pulse recording, Allen's testing and blood samples for determination of

erythrocyte sedimentation rate and antinuclear antibodies (ANA). Patients with significant positive ANA were excluded from the study. Also patients with a history of acute embolization to the brachial artery were not included in the material but operated on without prior investigations.

In the remaining 37 patients the suspicion of a stenosis or occlusion was not only based on their histories, physical examinations and blood samples, but also on the result from a recently developed finger pletysmographic method with local cooling (CCT) (16). These measurements were performed at rest and after vasodilatation by body warming (warming pad of 50°C over the abdomen for 15 min) and body warming in combination with 30 ml orally administered 40 % alcohol (CCT-WA) (1). By this technique the quotient between the finger systolic blood pressure (FSBP) and the arm blood pressure was calculated at rest, after body warming and after body warming in combination with alcohol. A constant low quotient (< 0.8) during these vasodilatating procedures ought to indicate an organic occlusion or significant stenosis proximal to the finger tourniquet. This CCT-WA technique cannot eliminate the rare non-sympathetically induced vasospasm which is not even released by alcohol. To this vasospastic group belong probably patients with heavy β-blocking therapy and patients with a vasospastic form of connective tissue disorder (2).

ANGIOGRAPHIC PROCEDURES

The patients were examined in a fasting state. Smoking was not allowed 6 hours before the investigation. The room temperature was 19-20°C. Ten patients belonging to group 1 (see below) were examined with meglumine metrizoate (280 mg J/ml). The other 42 patients were examined with metrizamide (300 mg J/ml), a contrast medium of low osmolality. The first three of these 42 patients were examined with both contrast media.

Catheterization was performed from the femoral artery. A pig-tail catheter (ID/OD 1.4/2.3 mm) with multiple side-holes was placed in the ascending aorta. Immediately after introduction of the catheter 3 000 IU of heparin was injected through the catheter. Aortography was then performed by injection of 40-50 ml of 60 % contrast medium at a rate of 25 ml/s. Eight films were exposed at a rate of 2 films per second in RAO and LAO projection, respectively, to exclude stenotic lesions at the origins of the brachiocephalic arteries. The catheter was then exchanged for a similar catheter shaped for selective work with only an end-hole and catheterization of the appropriate brachial artery was carried out. The tip of the catheter was usually placed distal to the axillary branches. Ten ml of contrast medium was injected at a rate of 7-8 ml/s and films were exposed over the upper and lower arm to cover the arterial phase. An appropriate delay of exposure for examination of the hand was calculated from these films.

Examination of the hand was made after injection of 20 ml of contrast medium in the brachial artery at a rate of 7-8 ml/s and 1 film/s was exposed during 10 s.

From the angiogram, the catheter-to-wrist (CWT) and the catheter-to-metacarpophalangial-joint-times were calculated as were the times before the metacarpal veins appeared. After the initial angiography had been performed, five different vasodilatating treatments were tested (see also Table 1).

Treatment I: An axillary blockade of the brachial plexus was performed by injection of 15-20 ml 2 % lidocain (Xylocain®, Astra). In three patients with insufficient effect of the axillary blockade a complementary supraclavicular blockade of the plexus was performed.

Treatment II: Half a mg reserpine (Serpasil®, Ciba-Geigy) dissolved in 10 ml NaCl was injected slowly intraarterially. Angiographies were performed 10, 20, 30 or 40 minutes after injection.

Treatment III: Two, 4 or 6 mg phentolamine (Regitin®, Ciba-Geigy) was injected after inflating a brachial tourniquet to 50 mmHg. Three minutes after the injection the pressure of the tourniquet was released whereafter the angiography was performed.

Treatment IV: Body warming was achieved by placing a warming pad of 50°C over the abdomen. The patient was covered with blankets. Angiography was performed after 15 min.

Treatment V: Thirty ml 40 % alcohol was given orally. Angiography was performed after 15 min.

Seventeen patients who first had a blockade of the brachial plexus constituted group 1 (A-C) while 16 patients who first had an injection of phentolamine constituted group 2 (A-D) and 19 patients who first were vasodilatated by body warming constituted group 3 (A-D, see Table I).

The effectiveness of the vasodilatating treatments were tested by local cooling induced by letting the patient hold the hand in a bowl with ice cubes for 5 minutes. An angiography was performed before and after this cooling procedure. If more than one vasodilatating treatment was tested in the same patient new angiographies were carried out and in most patients also angiographically controlled local cold-provocation.

RESULTS

Angiographies performed by meglumine metrizoate (10 patients in group 1) caused severe pain in the arm. The shift to metrizamide effectively eliminated pain, while the status of the circulation,

evaluated in the three patients examined by both contrast media, was the same.

Initial angiography

In the initial angiogram a stenosis of the subclavian artery was observed in four patients and three patients showed a high bifurcation with the radial artery branching already at the axilla. The CWT:s were registered in patients belonging to groups 2 and 3. The mean value was 10 s (range 2-20 s) and calculated as the time for the contrast medium to pass from the tip of the catheter to the radial artery at the wrist level. If the CWT was more than 12 s, vessels at the metacarpophalangial joint were never visualized. In nine patients there were no filling of contrast medium in the ulnar artery at the wrist level. The metacarpal veins could never be demonstrated in the initial angiogram.

Angiography after vasodilatating treatments

Group 1 (Blockade of brachial plexus). Patients placed in group 1 were those with the suspicion of an organic occlusive lesion based only on their histories and physical examinations. A pronounced vasodilatation of the digital arteries with good filling of the plexa of the fingertips and a CWT < 4 s was achieved in 8 of the 17 patients with no vasospasm occurring after local cooling. In the remaining 9 patients the dilatation was uneven but at its maximal

points comparable to the other 8 patients. These 9 patients also displayed vasospasm after local cooling. In 3 of these patients this cold-induced vasospasm was slight but general, while in 6 it was marked but regionally located.

Blockade of the brachial plexus in combination with intraarterially administered reserpine given after the blockade (see Table I) did not give any further vasodilatation according to the estimation from the angiograms performed 10, 20 or 30 min after the injection (group 1 C).

Group 2 (phentolamine). In this group comprising 16 patients different doses of the reversible α-blocking agent phentolamine were given in quantities up to 6 mg. No systemic side effects were registered. Two-4 mg phentolamine was given to 14 patients resulting in a change of the CWT from 11 s (range 4-20 s) in the initial angiogram to 4 s (range 3-6 s). This treatment gave a good dilatation of the arterial tree sometimes even with filling of the plexa and no vasospasm was induced after local cooling. Increasing the dose of phentolamine to 6 mg in 5 of the 14 patients did not further decrease the CWT. The remaining 2 patients who demonstrated severe vasospasm in the initial angiograms with a CWT of more than 18 s had only slight vasodilatation after 4 mg phentolamine (CWT 11 and 10 s, respectively). Since an increase of dose did not affect the CWT in other patients belonging to the phentolamine group these two patients received instead an additional blockade of the brachial plexus which resulted in a marked

decrease in CWT to less than 4 seconds with no vasospasm induced after local cooling (2 C). These two patients had a connective tissue disorder (see Fig 1).

Phentolamine in combination with intraarterially administered reserpine (group 2 D) did not give any further vasodilatation or any decrease in CWT as evaluated by angiography performed 10, 20 or 30 minutes after reserpine injections.

Group 3 (Body warming). Body warming markedly decreased the CWT from 9 s (range 2-20 s) in the initial angiogram to a mean of 5 s (range 2-9 s) and improved the visualization of the palmar vessels. Some vasospasm was always achieved after local cooling.

Body warming in combination with 4 mg phentolamine (group 3 A) decreased the CWT to a mean of 2 s (range 2-3 s), which was lower than the CWT in patients who only received 4 mg of phentolamine alone (group 2). No vasospasm was achieved after local cooling (see Fig 2).

Body warming in combination with orally administered alcohol (group 3 C) caused only a slightly increased vasodilatation compared to body warming alone.

Body warming in combination with reserpine injections (group 3 D) did not cause any further vasodilatation as compared to body warming alone as judged by angiography performed 10 or 20 min after the

reserpine injections. Forty min after the injection, however, a great improvement in vasodilatation was observed. The CWT:s, the filling of the digital arteries and the plexa were comparable to those observed on the angiogram 40 min after a blockade of the brachial plexus performed in patients belonging to group 1.

Outcome of selection procedure

Of the 15 patients in our study with the suspicion of an organic lesion based purely on the histories and a physical examination, angiography could only confirm the diagnosis in eight. In the 37 patients where the suspicion was based on the CCT-WA test, 33 were found to have a stenosis, occlusion or anomaly prior to the proximal phalanx.

Complications

No uncomfortable hematoma was registered at the puncture site of the femoral artery. Three patients had slight nausea, probably due to the contrast medium. One patient had urticaria. One embolus in a branch of the radial artery occurred, fortunately without any clinical significance. All patients found the blockade of the brachial plexus painful, and the totally anesthetized arm uncomfortable. Three of these patients also complained of pain at the injection site up to 6 months after the investigation.

DISCUSSION

Indications for angiography

Angiography of the upper extremity should, with a few diagnostic exceptions (11, 14, 26) mainly be restricted to be a preoperative descriptive examination of organic stenosis or occlusions. Thus, only areas involved for the decision concerning reconstructive vascular surgery are of importance to visualize. Optimal angiographic demonstrations of organic stenosis or occlusions can only be obtained if simultaneously present vasospasm is eliminated, whether this is sympathetically induced or not.

Selection of patients for a preoperative angiography can be made by history taking, a physical examination, selected blood samples and the CCT-WA testing procedure (see METHOD). In our study the diagnostic accuracy from this selection improved markedly by the addition of the CCT-WA testing procedure from 53 % to 89 %. The CCT-WA technique cannot, however, eliminate the rare cases with non-sympathetically induced vasospasm which is not even released by alcohol. To this group belong in our study patients with, for example, a vasospastic form of a connective tissue disorder probably SLE.

Angiographic method

Angiography was performed via the transfemoral approach in order to find proximal organic lesions and anomalies and enables an investigation of both arms. The known incidence of intimal lesions and local vasospasm at the puncture site when performing the angiography from the brachial artery gave further reason for this preference. The transfemoral approach requires a long catheterization path and causes a lengthy examination time. Heparinization of the patient during the investigation is thus important.

Healthy persons in normal room temperature do not show any basal vasospasm (23). Demonstration of vasospasm in the initial angiography therefore indicates a vascular disorder and the necessity for pharmacologically induced vasodilatation for proper visualization of the vessels. If the CWT in this initial angiogram was less than 4 s and there was filling of the proper digital arteries the patients had, by our definition, no vasospasm. The patients in our study who according to these criteria were without vasospasm had a history of a single trauma (knife, road accident, iatrogenic lesions). Apart from the traumatic arterial lesion, their vessels could therefore probably be regarded as normal. Subsequently they should not have needed any vasodilatating treatment during angiography.

Vasodilatating treatments

Body warming. Rösch et al (22) have showed a correlation in a healthy person between finger tip temperature (increased by body warming) and the CWT calculated from angiograms (22^{o}-15 s, 33^{o}-3 s). Several investigators have found that, a normal blood flow is indicated by finger tip temperature of $32^{o}C$ or higher (19). Eighty per cent of the patients in this study had a mean finger tip temperature of more than $32^{o}C$ after body warming for 15 min as presented elsewhere (2). In this present study a mean CWT of 9 s in the initial angiogram is decreased to 5 s after body warming for 15 min. From these studies can be concluded that body warming in most patients causes an increase in skin temperature to 32^{o} or higher which angiographically can correspond to a CWT of approximately 3-5 s and to normal blood flood. Body warming thus seems to be a comfortable and simple method to achieve a good vasodilatation.

Phentolamine is a reversible α-blocking agent. It has a short acting time of 10-15 min. A maximal effect is to be expected 3-5 min after the injection. In our study 4 mg phentolamine gave an optimal vasodilatation without side effects. If only a slight vasodilatating effect and the CWT > 10 s are achieved after 4 mg phentolamine another vasodilatating treatment ought to be considered. The poor response to phentolamine can of course be a question of dosage to release the severe vasospasm, but can also be explained by a non-sympathetically induced vasospasm. Noticeable is the complete release of this kind of vasospasm after blockade of the brachial plexus.

Peacock and Zeitler have in separate studies pointed out the importance of using two vasodilatating treatments in combination to achieve a maximal effect (18, 31). We can here confirm their statements. In our study body warming in combination with 4 mg phentolamine intra-arterially gave a more pronounced vasodilatation and significantly lower CWT (2 s) corresponding to a normal blood flow (see Discussion above) in patients with sympathetically induced vasospasm than either of these treatments alone.

Orally administered alcohol in combination with body warming is in our present study not superior to body warming in combination with phentolamine injections. This is in variance with the results from Zeitler (30) who reported a marked vasodilatating effect after orally administered alcohol though the given dose is not reported. This discrepancy can perhaps be explained by our preset body warming or be a question of dose.

Injection of reserpine intra-arterially induces an initial vasospasm by the momentary release of noradrenalin from the presynaptic terminals. This reaction which occurs before vasodilatation is achieved, has given cause to regard this drug as being less useful in angiography. Paolino (17) has demonstrated a increase in filling of the hand arteries already 15 min after a local injection of 1 mg of reserpine but other investigators have preferred to perform angiography 48 hours after intra-arterial injection of reserpine with good vasodilatation but still with some cold induced vasospasm (23).

We have previously reported that 0.5 mg reserpine intra-arterially gave a clinically noticable vasodilatation within a few hours and a maximal response after 24 hours (3). The present study showed that the vasodilatating effect of reserpine started after about 35 min and with a further increase after 40 min. In the angiograms performed 40 min after injection of 0.5 mg reserpine the CWT and the visualization of the digital arteries corresponded well to those obtained 40 min after blockade of the brachial plexus. The disadvantages of a blockade of the brachial plexus, i.e. being painful, time-consuming and difficult to get complete, should be taken into account before a decision is made concerning these two treatments, especially as the degree of vasodilatation probably is the same.

We conclude that body warming in combination with an injection of 4 mg of phentolamine by eliminating sympathetically induced vasospasm give a normal blood flow and sufficient vasodilatation for visualization of the pertinent areas for a decision about reconstructive vascular surgery. This investigation procedure is comfortable for the patient and the activity of the drug is brief. Body warming in combination with injection of 0.5 mg of reserpine give a more pronounced vasodilatation in the periphery but the investigation time is increased and the effect of the vasodilatating drug long-lasting (one week). Blockade of the brachial plexus eliminates the rare cases of vasospasm which were not markedly affected by phentolamine.

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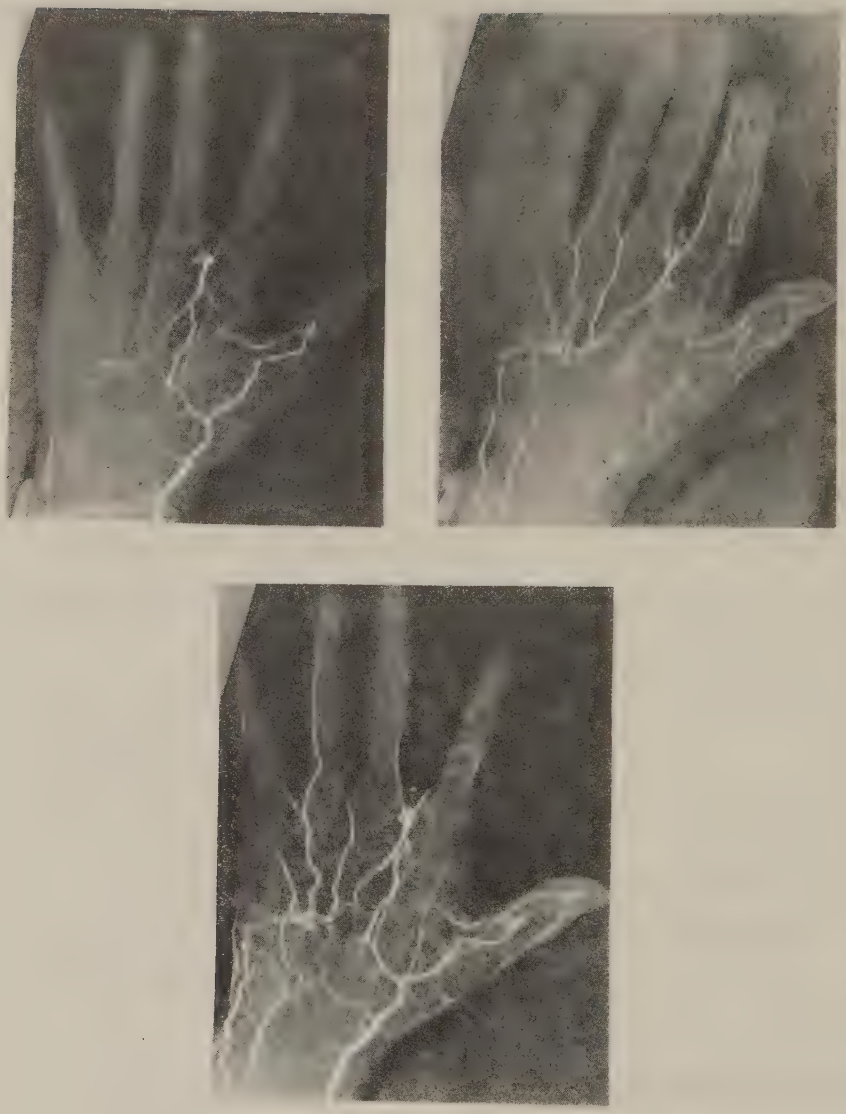

Fig 1. Angiograms before vasodilatation (A), after body warming (B) and after injection of 4 mg of phentolamine (C).

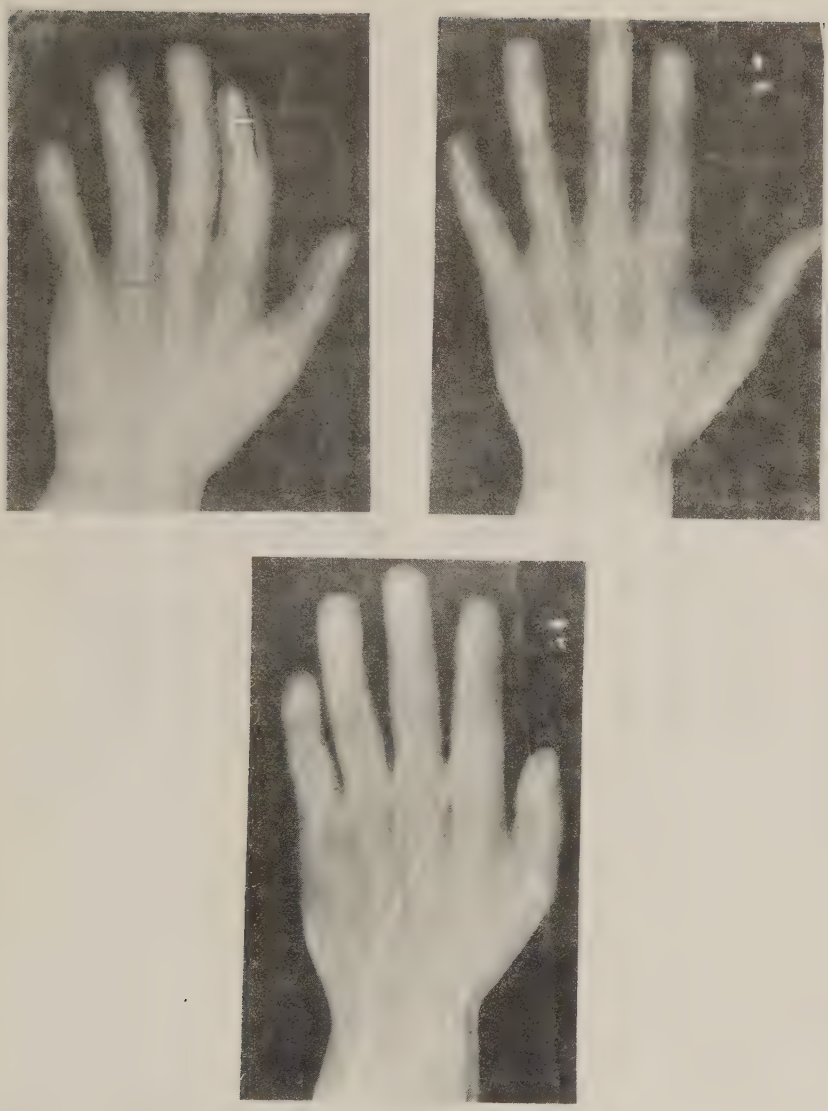

Fig 2. Angiograms before vasodilatation (A), after injection of 4 mg of phentolamine (B) and after blockade of the brachial plexus (C).

Table I. Vasodilatating treatments in 52 patients with Raynaud's phenomenon.

Group	n	Treatment
1 A	9	Blockade of the brachial plexus + local cooling
1 B	4	Blockade of the brachial plexus
1 C	4	Blockade of the brachial plexus + local cooling + reserpine
2 A	6	Phentolamine + local cooling
2 B	5	Phentolamine
2 C	2	Phentolamine + local cooling + blockade of the brachial plexus + local cooling
2 D	3	Phentolamine + local cooling + reserpine
3 A	9	Body warming + local cooling + phentolamine + local cooling
3 B	4	Body warming + phentolamine
3 C	4	Body warming + local cooling + orally administered alcohol + local cooling
3 D	2	Body warming + local cooling + reserpine

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BIRGITTA ARNEKLO NOBIN, M.D., STEEN LEVIN NIELSEN, M.D., BO EKLØV, M.D., NIELS A. LASSEN, M.D.

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Reserpine Treatment of Raynaud's Disease

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Six patients with primary Raynaud's disease were investigated with magnification hand arteriography, measurement of finger systolic pressure and response to a newly devised local cooling test. They were treated with reserpine 0.5 mg injected into the brachial artery, and the clinical effects were followed by a scoring system and by the change in digital arterial tone during local cooling. The latter was registered as the change in systolic finger blood pressure measured indirectly by a finger-plethysmograph during stepwise decrease in finger temperature, until at a critical temperature complete closure of the digital arteries occurred. Shortly after injection the clinical condition improved, and closure of the digital arteries during local cooling was impeded. But within a week the cooling test showed a transient deterioration with closure of the digital arteries at the same or a somewhat higher temperature than before the reserpine injection. The clinical score followed this trend. According to animal experiments the shortlasting vascular effect of reserpine on primary Raynaud's disease might be due to depletion of the sympathetic nerve terminals for norepinephrine.

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IN RECENT YEARS, reserpine administered perorally or intra-arterially has been claimed to be the most efficient drug treatment of the condition named primary Raynaud's disease.[1,2,5,11,12] The reported clinical effects are, however, diverging both as to relief of symptoms and to duration of the effect varying from few days to several months. The pathogenetic mechanism behind the Raynaud attack provoked by cold exposure is only partly understood.[2]

From the experimental studies performed some 50 years ago, Sir Thomas Lewis concluded that the clinical entity described by Maurice Raynaud in 1862 is basically caused by a complete closure of the digital arteries as a local response of the vascular smooth muscle to finger cooling.[4]

Closure of the digital arteries is a prerequisite for the 'doigt mort.' But sympathetic nervous activity is, at present, considered to be just as important as the local myogenic reaction.[6] This is the background for many proposed medical treatments comprising a variety of blocking drugs, and surgical intervention performed as sympathectomy in severe cases.[2] The effects of these treatments are, however, often discouraging with only transient clinical improvement and many side effects. The present study deals with the clinical and experimental evidence for the effect of reserpine given intra-arterially to patients with primary Raynaud's disease of varying severity as judged from arteriography and clinical symptoms. A newly developed method for measurement of digital arterial tone and closure temperature[7] allowed us to follow the acute and prolonged effects of reserpine treatment in a quantitative way.

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Material and Methods

The patient material consists of six female patients, (mean age 37), referred to the department of vascular surgery for Raynaud's disease with a duration of more than five years (Table 1). They were normally menstruating, taking no vasoactive drugs or antiovulatory drugs. They all went through a thoroughly clinical examination comprising exact information on provoking factors and progression of the disease. Routinely venous blood samples were taken for analyses of hemoglobin thrombocytes, sedimentation rate, cryoglobulins, and cold agglutinin. Thyroid hormones (T_4 and TBG_m), immunological factors (AST, Latex fixation, Waaler-Rose reaction), and coagulation mechanism (fibrinogen, fibrinolysis and coagulation time) were analysed. X-ray of neck excluded cervical ribs.

During hospitalisation arteriography was performed as a combined aorto-cervical angiogram excluding proximal stenosis and a selective brachial arteriography demonstrating the small finger arteries by magnification. The latter was repeated after cooling of the hand by crushing an icecube for some minutes, and in three cases half an hour after injection of 0.5 mg reserpine through the catheter positioned in the brachial artery. The arteriograms were evaluated

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TABLE 1. *Clinical Data on Six Female Patients with Primary Raynaud's Disease for More than Five Years*

No.	Age	Blood pressure		A-graphy	Clinical score			Closing temperature			
		arm	finger		2–4 h	3–6 d	28 d	0	2–4 h	3–6 d	28 d
1	42	120/80	96	spasm/ stenosis	3	−1	0	21.5	16	22	19.5
2	36	100/68	98	spasm/ stenosis	1	0	−1	14.5	<10	17	20.5
3	33	124/70	35	spasm/ stenosis	5	0	—	15.0	<10	20.5	—
4	41	100/64	68	spasm/ stenosis	3	—	−1	23	16	—	19
5	37	110/80	42	spasm/ stenosis	3	—	6	<10	<10	—	15
6	32	105/70	90	spasm/ stenosis	1	1	0	<10	<10	<10	14.5

blindly by an experienced radiologist for organic stenoses or occlusions. The response to cold was taken as indication of a vasospatic moment (Table 1).

Fingersystolic blood pressure was measured using a specially devised plastic cuff on the midphalanges of ulnar fingers.[3] The volume increase on the outer phalanx when deflating the cuff was recorded by a mercury-in-silastic strain gauge. The finger exhibiting color changes most frequently was thereafter studied applying local cooling (Fig. 1). Finger systolic pressure was measured with a double inlet cuff before and after direct cooling of the midphalanx for periods of five minutes with circulation stopped by a tourniquet on the proximal phalanx. The temperature was lowered stepwise by 2.5–5° per step down to a level of 10°. At each temperature level finger systolic pressure was determined repeatedly until it regained the control level due to rewarming by the blood. Systolic blood pressure in the supplying arteries was estimated by measurement of finger systolic pressure in a control finger on the same hand. Arm systolic blood pressure was determined repeatedly to exclude changes in systemic blood pressure as measured by the conventional auscultatory technique. Temperature on fingers, in rooms and cooling devise was measured by thermocouples.

The cooling test was performed with the lightly dressed patient supine after a one half hour rest with room temperature at 23–25°. The patients were relaxed and sometimes fell asleep indicating absence of physical discomfort or distress. After initial investigation, 0.5 mg reserpine (Serpasil®, CIBA) diluted in 10 ml saline was given in the brachial artery. The injection was made in local anesthesia by direct puncture with a 21 gauge needle, the injection being performed in two to three minutes. Measurement of finger systolic pressure and reaction to local cold was repeated two to four hours after injection, three to eight days later and finally after 25–28 days. Information about the subjective effect was reported every second day in the ambulatory patients and clinical improvement of the injected hand was recorded by a scoring system (Table 2).

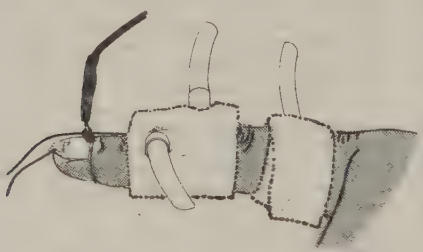

FIG. 1. Application of a blood pressure cuff on middle phalanx for local water cooling and measurement of systolic finger blood pressure by registration of a volume signal with a mercury-in-silastic strain gauge. During cooling the small cuff located on the proximal phalanx was used as tourniquet.

Results

During local cooling of the midphalanx a decrease in finger systolic pressure is observed indicating an in-

TABLE 2. *Clinical Scoring System for Complaints in Raynaud's Disease*

Complaint	Score			
	2	1	0	−1
Raynaud's phenomenon	absent	less frequent	unchanged	more frequent
Finger/hand temperature as compared to opposite		warmer	equal	cooler
Cyanosis of fingers	absent	decreased	unchanged	worsened
Tenderness of fingers	absent		unchanged	increased
Finger sores	healed		unchanged	worsened

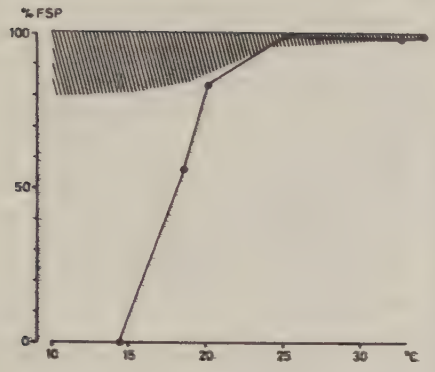

FIG. 2. Response to local cooling in a patient with primary Raynaud's disease measured as the percentage change in finger systolic blood pressure (FSP) during local cooling of the midphalanx of the right third finger. The variation in decrease of FSP during finger cooling in 15 normals are given by the hatching with 2.5 SD as lower limit.

crease in digital arterial tone (Fig. 2, case no. 2). It is seen that the arterial tone shows a gradual increase being significant compared to the normal group at 18.5° (threshold temperature) and leading to a sudden increase in tone giving complete closure of the digital arteries on the midphalanx at 14.5° (closure temperature). The hand arteriography for this patient is given in Figure 3.

For the six treated patients alterations in closing temperature and clinical score is given in Figure 4. The hand and forearm became pale eventually with pains immediately after the injection. At investigation two hours after injection, the hands were red, warm and sometimes a little edematous. Two patients (case 2 and case 3) had only clinical effect for about two days, the others had it for six to eight days (with the exception of case no. 5, who had only minor complaints of recurrence after four weeks). This patient had ulcers and tender fingertips before the injection as her main complaint, and the improvement was due to healing of the ulcers. She had been bilaterally sympathectomised three years earlier, and had only few Raynaud attacks.

In accordance with the clinical scoring the local cooling test showed an initial improvement after reserpine injection appearing as decrease in closing temperature. Within a week deterioration was observed in all cases by an increase in closing temperature and changes in threshold temperature followed this trend. Corresponding to the changes in closing temperature the digital arterial tone measured at 20° showed an initial decrease after reserpine injection followed by an increase to the control level (Fig. 5). This indicates that

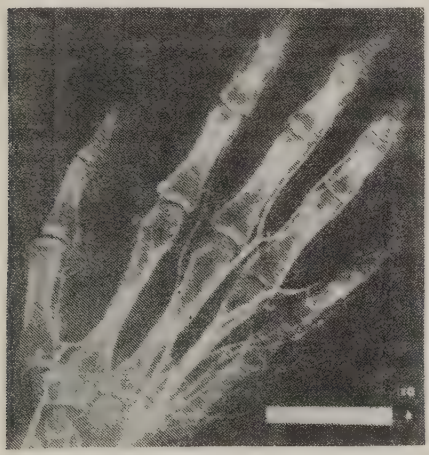

FIG. 3. Magnification hand arterigram from patient in Fig. 2. Note obliterations in the digital arteries.

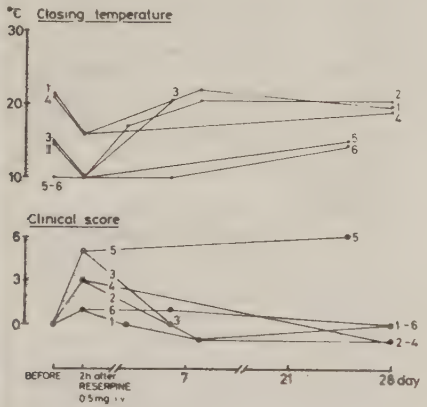

FIG. 4. Effect of reserpinisation in six patients with primary Raynaud's disease expressed as response to local cooling on closing temperature and the clinical score (Table 2).

the patients at room temperature will only benefit from injection of reserpine for less than a week by a lowered arterial tone.

Discussion

The presented measurements of the effect of reserpine on patients with Raynaud's disease have several important implications. The techniques used were hand arteriography and measurement of digital arterial tone during local cooling. The first method can demonstrate arterial obliterations, but as all contrast medias are more or less vasodilating, only measurements during local cooling can give clue to diagnosis of a 'vasospastic moment' in the disease. Measurements of digital arterial tone showed a decreased response of the digital arteries to local cooling for one to eight days after intraarterial injection of 0.5 mg reserpine with a maximum effect for one to two days. The objective measures followed closely the clinical effects registered by a score system, except in a patient showing sore healing.

There is ample evidence from animal experiments that the acute effects of intra-arterial reserpine administration is caused by a more or less complete and reversible depletion of sympathetic nerve endings for norepinephrine.[9] The norepinephrine release can initially give a pronounced vasoconstriction, but this is within some minutes to hours followed by a local vasodilatation with partial or complete absence of sympathetic tone and blocked response to sympathetic stimuli. The effects are maximal for one to two days and subside thereafter gradually eventually followed for a short period of hypersensitivity to norepinephrine and sympathetic stimulation. The generally held view that this time course is due to norepinephrine depletion of the sympathetic vesicles has been confirmed by histological studies on different tissues of the catecholamine stores.[9]

The time course for the action of reserpine resembles completely the clinical and experimental findings in the present study. This indicates that the direct pharmacological action of reserpine given as an intra-arterial injection is of short duration (from one to eight days). Reported effects of reserpine in Raynaud's disease vary both as to positive response and to duration of relief of symptoms. This could partly be explained by inhomogeneity of the patient materials because both primary and secondary forms of Raynaud's phenomenon are included. Long lasting effect of intraarterial reserpine for several months[1,12] seemed to be due to healing of ulcers and improvement of tender fingertips.

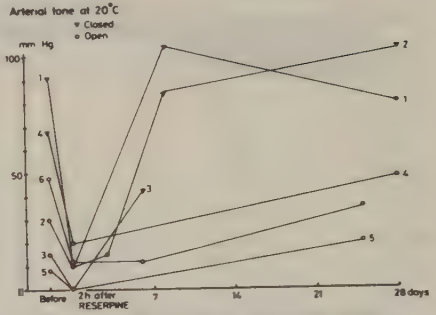

FIG. 5. Digital arterial tone estimated indirectly on fingers cooled to 20°C in six patients with primary Raynaud's disease, before and after reserpine injection.

The short term effects measured by changes in skin blood flow,[11] and on clinical parameters in a study with control injection in the opposite arm[5] had a duration of few days. The divergences are not incompatible, since sore healing only takes few days if circulation is improved shortly by the chemical sympathectomy caused by reserpine. If the sore fingertips are healed and then carefully protected they can be kept in a reasonably good condition for a long time. Such severe cases of Raynaud's disease might be the only indication for intra-arterial reserpine administration.

In a period with decreased response of the digital arteries to external cold attacks Raynaud's phenomenon will be less frequent and probably of shorter duration. If the hand temperature furthermore is elevated the cooling time necessary to reach the well defined closing temperature will be increased. Clinical observations are in agreement with these predictions.[1,5,11,12] However, the relatively short duration of the reserpine effect (one to eight days) reserves intra-arterial treatment with reserpine to the severe cases.[2] Not only is there a risk by repeated intra-arterial injections that should be given weekly, but reserpine has also been reported to be associated with development of breast cancer.

From the present study we conclude that the action of intra-arterial reserpine in humans corresponds to earlier findings in animal experiments. Beneficial effects for more than a few days are due to healing of ischemic lesions. Therefore therapy with reserpine should only be considered in severe cases of Raynaud's disease where amputation and sympathectomy might be postponed.

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